Chapter 3

EFFECTS OF CHANGES IN FOREST USE ON BIODIVERSITY

Chapter 3 Introduction

Satoshi Yamashita
Research Institute for Humanity and Nature

In Chapter 2, the changes in forest utilization during the past 50 to 100 years were revealed for each study site. Based on this understanding, we will discuss the effects of forest utilization on the biological community at each study site in section 3.1 and the effects of human activities on ecosystem functions in section 3.2.

In section 3.1, we discuss the effects of forest utilization on communities or populations of living organisms at each study site. At Lambir, species diversity and the species composition of plants (Momose *et al.*), insects (*i.e.* butterflies (Itioka *et al.*), beetles (Kishimoto-Yamada *et al.*), ants (Matsumoto *et al.*)), mammals (*i.e.* bats (Fukuda *et al.*), small mammals (Nakagawa *et al.*)), and macrofungi (Yamashita *et al.*) were compared among several forest types (*i.e.* rubber plantation, secondary forest after swidden agriculture, isolated natural forest, and primary forest). In every biological community except for the beetle community, species diversity was highest in the primary forest and generally decreased with increasing disturbance of the forest. At Sabah, species diversity and the species composition of plants (Aiba *et al.*), insects (*i.e.* flies (Akutsu *et al.*)), soil animals (Ito *et al.*) and mammals (Onoguchi & Matsubayashi) were compared between forest managed under reduced-impact logging, forest managed under conventional logging, and primary forest. Species diversity of plants and mammals was higher in forest managed under reduced-impact logging than in forest managed under conventional logging methods. On Yaku Island, the species diversity and biomass of plants (Aiba *et al.*, Tsujino *et al.*) and insects (*e.g.*, beetles, horseflies, wasps (Yamauchi *et al.*)) and the density of the Japanese macaque (Hanya *et al.*), were surveyed in primary forest, plantations of Japanese cedar, and secondary forest. Species diversity of plants and insects was lower in the plantation forest than in the primary forest. At Abukuma, the community structure of the forest-floor vegetation (Tanaka *et al.*, Nagaike *et al.*) and of arthropods (*e.g.*, butterflies, moths, wasps, beetles, springtails, mites (Makino *et al.*)) was surveyed in several different forest types (*e.g.*, plantations of Japanese cedar and secondary and old growth broadleaved deciduous forest) for a range of forest ages. Species diversity of the forest-floor vegetation was higher in young stands than in intermediate-aged and old stands in both the secondary forest and the plantation forest. Species composition differed among the forest types. Arthropods could be divided into three groups based on the response of their species diversity to the age of the forest: in one group, species diversity decreased with increasing forest age (*e.g.*, butterflies, longhorn beetles); in the second group, species diversity increased with increasing forest age (*e.g.*, mycophagous mites); and in the third group, species diversity did not change with increasing forest age (*e.g.*, moths, ground beetles).

In section 3.2, we discuss the effects of human activities on ecosystem functions. Here, we define ecosystem functions as the functions that are necessary for the growth or sustenance of the ecosystem, such as pollination, the food chain, and matter and energy flows. Recently, the area of forest has decreased rapidly, accompanied by fragmentation of large stands into smaller stands, as was seen in Chapter 2. We hypothesized that fragmentation of forests would reduce the size of plant populations, leading to reductions in visitation by pollinators, reductions in the number of individuals capable of reproduction, and inbreeding depression. In section 3.2.1., we discuss the results of some basic studies of plant ecology at Lambir (Aiba &

Nakashizuka, Kenzo *et al*., Ichie *et al*.) and Yaku island (Tanabe *et al*.). In section 3.2.2., we introduce studies on plant reproductive ecology (Momose *et al*.) and plant–pollinator interactions (Kumano & Yamaoka, Ushimaru *et al*.), and then we discuss the effects of forest fragmentation caused by human activities on plant community. Studies of one tree species at Lambir (Takeuchi *et al*.) and two tree species at Abukuma (Matsuki & Isagi, Tateno *et al*., Kikuchi *et al*., Shibata *et al*.) revealed that forest fragmentation does not always have a negative influence on tree reproduction.

In section 3.2.3., we discuss the results of studies of changes in seed-dispersal systems in response to changing human utilization of the forest. At Lambir, the pattern of fruit utilization by bird communities is described (Kamoi *et al*.). At Sabah, the loss of large mammal seed-dispersers after conventional logging appeared to have detrimental effects on the regeneration of two *Durio* species (Nakashima & Matsubayashi). On Yaku Island, the restoration of a *Ficus* species population after logging conducted 60 years ago is not yet complete (Otani & Sei-ichi). The behavior of the Japanese macaque determines the spatial distribution of the tree *Myrica rubra* (Terakawa *et al*.), and seeds of *M. rubra* was attacked by lepidopteran insects before dispersal (Fujita *et al*.). At Abukuma, forest fragmentation positively affected the growth of seedlings of plant species that are dispersed by birds (Naoe *et al*.).

The second and third sections of this chapter show that the effects of human activities on plant regeneration are highly variable. The effects on species interactions might affect plant–seed disperser systems, plant–pollinator systems, or some other combination of these systems described in section 3.2.4. (Tanaka *et al*., Handa *et al*., Tzuchiya & Itioka, Okubo & Itioka). At Lambir, swidden cultivation reduced the species diversity of ants and plants, which means that simplification of the species composition also simplified the interspecific interactions, and the effects were still detectable at least 20 years after the initial disturbance (Tanaka *et al*.).

Some studies of the effects of human activities on interspecific interactions have pointed out that human activities sometimes affects these interactions and that the response of the system differs depending on a range of factors. Such changes in community structure might affect matter and energy flows through the forest ecosystem. In section 3.2.5., we focus on matter flows, especially through the decomposition system (Wagai *et al*.). At Sabah, the decomposition rate of leaf litter was highest in the forest managed under conventional logging (Hasegawa *et al*.).

In this report, we will present an assessment method for evaluating the level of sustainable forest use. The results permit an assessment of the ecological situation at the study sites. The results clearly demonstrate the different responses of species diversity of various organisms and of ecological functions to human activities, and suggest that understanding the traits of individual organisms is needed before evaluation can occur.

Effect of Forest Use on Microhabitat Environment and Vegetation Structure in Sarawak, Malaysia

Kuniyasu Momose[1], Michi Kaga[2], Miyako Koizumi[2], Keiko Kishimoto-Yamada[3], Hiroshi O Tanaka[3], Takashi Matsumoto[3], Takao Itioka[3], Michiko Nakagawa[4], Masahiro Ichikawa[5], Mitsunori Yoshimura[5], Tohru Nakashizuka[6], Lucy Chong[7]

[1] *Faculty of Agriculture, Ehime University*

[2] *Graduate School of Asian and African Area Studies, Kyoto University*

[3] *Graduate School of Human and Environmental Studies, Kyoto University*

[4] *Graduate School of Bioagricultural Sciences, Nagoya University*

[5] *Research Institute for Humanity and Nature*

[6] *Graduate School of Life Sciences, Tohoku University*

[7] *Sarawak Forestry Corporation, Malaysia*

Introduction

Tropical rain forests in Southeast Asia have been exposed to drastic and rapid deforestation because of an increasing human population and a rapidly developing economy. The annual deforestation rate in this region is 1.9%, whereas the average global rate is only 0.2 to 0.3% (Matthews 2001). Elucidation of the effects of forest use on plant biodiversity and on the microhabitat environment is an urgent subject for species conservation and proper forest management. Many studies have been performed on the effects of forest fragmentation on changes in tree species composition or abundance (e.g., Laurance et al. 2000). These studies have revealed that the structure of a forest remnant might be affected by its area, its distance from primary forest, and the elapsed time since fragmentation began (Laurance et al. 2002).

In Southeast Asia, forests are used for various purposes, including traditional ones. Slash-and-burn agriculture remains a widespread method of cultivation; local people (the Iban) abandon a field and shift to a new site after harvesting. As a result, secondary forests (fallows) at various developmental stages form a mosaic of landscape. Because rubber trees have been planted in abandoned fallows, particularly since the 1950s to produce crude rubber as a cash crop in Sarawak, Malaysia, rubber plantations operated by small holders are another widespread vegetation type (Ichikawa 2003). To understand the effect of forest use on a region’s vegetation structure, research must be conducted on the diverse vegetation types that form the forest mosaic. The alternation of vegetation structures may also be accompanied by changes in the microhabitat environment, such as canopy openness and soil-water content (Beaudet and Messier 2002). However, the effect of forest use on the microhabitat environment of various vegetation types remains unknown.

The objective of the present study was thus to compare the microhabitat environment and vegetation structure in fragmented primeval forests, abandoned fallows at three developmental stages, and rubber plantations, with those of primary forests so as to determine the effects of human forest use on the ecological traits of the vegetation in Sarawak, Malaysia. For fragmented primeval forests, the effects of the area, distance from a primary forest, and elapsed time since the fragmentation on vegetation structure were also examined.

Materials and methods

Study site and vegetation structure

A field survey was conducted in August 2003 in and around Lambir Hills National Park (LHNP), Sarawak, Malaysia (4°2’N, 113°5’E). The study site contained 32 plots (each 10×100 m) in six forest types: primary forests (four plots); fragmented primeval forests (nine plots); abandoned fallows at three developmental stages (i.e., new, 1 year after abandonment, four plots; young, 5 to 6 years after abandonment, four plots; and old, > 20 years after abandonment, six plots); and rubber plantations (five plots).

All trees in each plot ≥10 cm diameter at breast height (DBH, 1.3 m above ground) were tagged, mapped, measured DBH to the nearest mm, and identified to the species level. Voucher specimens were stored in the Herbarium of the Forest Research Center, Sarawak. Digitized satellite image was also used to identify the location of each plot, measure the shortest straight distance from the edge of each fragmented primeval forest plot to the primary forest of the park, and calculate the area of the fragmented primeval forest plots (Fig. 1). Since the exact date of fragmentation for each fragmented primeval forest was unknown, to estimate the date of fragmentation, we used the area of surrounding primary-forest-like vegetation within 200 m of each study plot calculated using a digitized aerial photograph of the region taken in 1977. The surrounding vegetation was classified into seven types: secondary forest (rubber plantation and fallow), open land (roads and grassland), wetland (wet rice fields and ponds), oil palm plantation, fragmented primeval forest, logged forest, and primary forest. The primary-forest-like vegetation included fragmented primeval, logged, and primary forest.

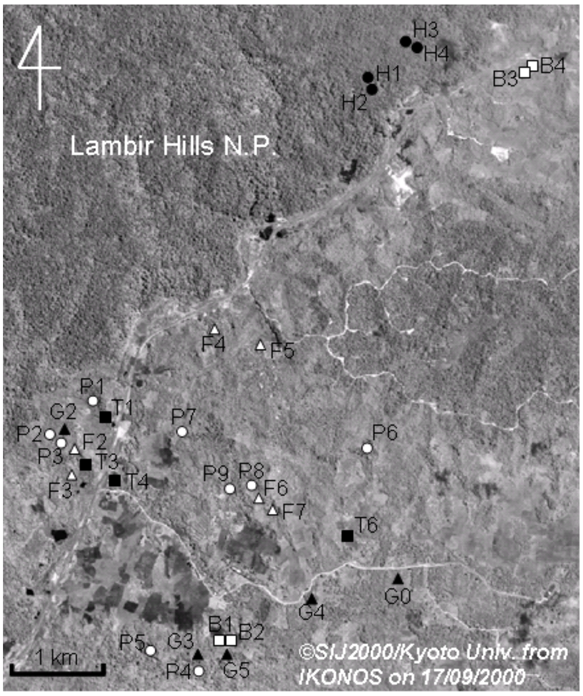


Fig. 1 Location of the study plots in and around Lambir Hills National Park, Sarawak, Malaysia. Symbols indicate the vegetation types: solid circle, primary forest; open circle, fragmented primeval forest; solid square, young fallow; open square, new fallow; solid triangle, rubber plantation; open triangle, old fallow. See Table 1 for plot codes.

Microhabitat environment

To characterize the light conditions at the forest floor, we measured the percent canopy openness using a digital camera with a fisheye lens (CoolPix 910, Nikon). Ten images were taken from the ground in each plot at 10-m intervals and analyzed using the CanopOn2 software (CanopOn 2003).

The soil-water potential was quantified using the method of Deka et al. (1995) in each study plot. Soil samples were taken on 20 February 2005, after a relatively dry spell during which no rain fell for six consecutive days. We sampled 10 soil cores to a 10-cm depth (3-cm in diameter) at 10-m intervals after removing the surface litter. Each soil sample was sealed in a plastic box for 8 days with filter paper (No. 42, Whatman) placed in the middle of the soil. On 1 March 2005, the filter paper was then weighed to within 1 mg after quickly removing the soil with a small paintbrush, and the soil-water potential was calculated (Deka et al. 1995).

Data analysis

We calculated the Shannon-Wiener *H’* parameter as a species diversity index using basal area data for each plot. Analysis of variance (ANOVA) was used to compare the effects of forest use on vegetation structure and microhabitat environment among the vegetation types, after log (absolute values) or arcsine (percentage values) transformation. For plots of fragmented primeval forest, we evaluated the relationships between vegetation structure traits (density, number of species, basal area, and H’) and the area, distance to primary forest, or area of surrounding primary-forest-like vegetation within 200 m of each study plot using a Pearson’s correlation coefficient. All statistical analyses were performed using the JMP 6.0 software (SAS Institute 2005).

Results

Vegetation structure and microhabitat environment

Since no trees ≧10 cm in DBH were found in the new fallow plots, we excluded this category from the ANOVA for vegetation structure. All variables that described vegetation structure and microhabitat environment differed significantly among vegetation types (Table 1). Primary forest and fragmented primeval forest had similar vegetation structures and dominant trees (Dipterocarpaceae), and they showed high tree density, number of species, basal area, and *H’*. Young fallow and rubber plantation plots had significantly low number of species and *H’*, though the density and basal area in young fallow were similar to those of old fallow, which had intermediate vegetation structure traits. The dominant trees were *Vitex pinnata* (Verbenaceae) in young fallow plots, *Artocarpus elasticus* (Moraceae) in old fallow plots, and *Hevea brasiliensis* (Euphorbiaceae, rubber tree) in rubber plantation plots (Table 1). In new fallow plots, grasses (Cyperaceae) grew thickly, mixed with small *Macaranga* and *Artocarpus* trees. Canopy openness and soil-water potential were highest in new fallow and fragmented primeval forest, respectively.

Table 1 Vegetation structure and microhabitat environment of six vegetatoin types studied in and around Lambir Hills National Park. Average variables of each vegetation type were shown.

Vegetation type (plot code)	*N*	Density (/0.1ha)	Species no (/0.1ha)	BA (m^2/ha)	H'	Canopy openness (%)	Water potential	Dominant tree[a]
Primary forest (H)	4	58	44.0	43.1	3.2	7.3	1.5	Dipterocarp trees
Fragmented primeval forest (P)	9	70	47.0	40.1	3.0	7.9	2.4	Dipterocarp trees
New fallow (B)	4	-	-	-	-	20.5	1.9	-
Young fallow (T)	4	40	15.5	7.9	2.0	9.8	1.9	*Vitex pinnata*
Old fallow (F)	6	53	32.7	20.9	2.8	8.2	1.9	*Artocarpus elasticus*
Rubber plantation (G)	5	60	19.2	20.6	1.8	10.8	1.9	*Hevea brasiliensis*

[a] Based on basal area.

Fragmented primeval forest

No significant correlations between forest area and vegetation structure were found for fragmented primeval forest. Tree density and species richness were significantly and negatively correlated with the distance to primary forest (Fig. 2). In contrast, we found significant and positive correlations between the area of the surrounding primary-forest-like vegetation within 200 m of each plot and tree density, species richness, and basal area.

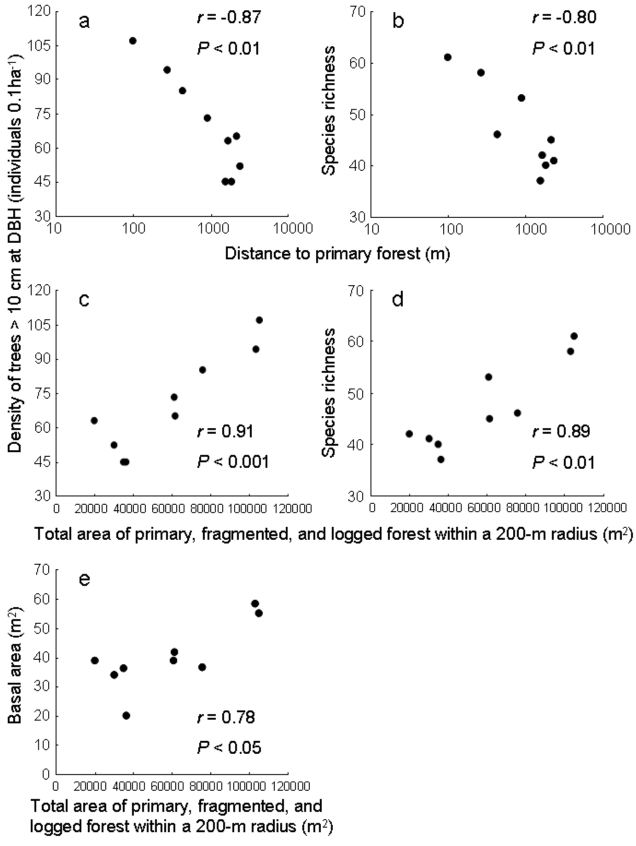


Fig. 2 Correlations between distance to primary forest from each fragmented primeval forest plot and (a) tree density (individuals 0.1ha^{-1}) and (b) species richness (species numbers 0.1ha^{-1}), and between area of surrounding primary-forest-like vegetation within 200 m of each fragmented primeval forest plot and (c) tree density, (d) species richness, and (e) basal area (m^2 ha^{-1}).

Discussion

Characteristics of the vegetation structure and microhabitat environment differed obviously among the vegetation types. Primary forest and fragmented primeval forest had larger and denser trees, with higher species richness and a darker forest floor (i.e., reduced canopy openness). However, the soil-water potential was highest in fragmented primeval forest. This might be because these forests are located mainly on ridges. Old fallow had the third-highest species richness, though the trees were smaller than those of primary forest or fragmented primeval forest and the dominant trees were also different. The differences in density and basal area between the young fallow and rubber plantation plots may have been caused by the planting of rubber trees. The vegetation structure and canopy openness in new fallow were distinctive, our results indicate that trees were able to recover from the disturbance and begin growing again relatively quickly, within several years after slash-and-burn agriculture. The alternation of the vegetation structure and microhabitat environment might affect not only the regeneration success of plants and forest dynamics (Bruna 1999; Cascante et al. 2002; Takeuchi et al. unpublished data) but also the biodiversity of fauna and the interactions between plants and animals (Medellín and Equihua 1998; Ferraz et al. 2003; also see several papers in this report).

Although species-area relationships have been widely used both to predict the effects of habitat loss on extinction rates and to guide conservation design (e.g., Wilson 1989), we observed no effects of the area of remnant forest on vegetation structure. Tree density, species richness, and basal area were instead affected by the characteristics of the surrounding vegetation, and particularly by the distance to primary forest and the area of surrounding primary-forest-like vegetation. This indicates that a small-scale mosaics composed of a range of forest types may be important because it enables trees to regenerate or immigrate to neighboring remnants by means of seed dispersal, thereby mitigating otherwise drastic habitat alterations. Gascon et al. (1999) also suggested the importance of a matrix of modified habitats surrounding forest fragments in terms of its effects on the dynamics or composition of vertebrate communities in tropical forest remnants. To practice proper land-use management, it will thus be necessary to consider the distribution of various vegetation types at the landscape level.

Acknowledgments

We thank L. Chong (Sarawak Forestry Corporation) and J. Kendawang (Forest Department Sarawak) for their permission to conduct research in Sarawak and their assistance with the necessary arrangements. The hospitable support and corporation of the inhabitants at Rh. Aji, Rh. Bundan, and Nakat was very helpful. Our field survey was kindly supported by S. Tsuji, M. Aiba, Jugok, Jinggan, Redtila, Akam, Jugok, Umping, Stewart, Douglas, and Maok. Financial support was provided by RIHN research project 2-2 and JSPS Research Fellowships for Young Scientists to M. Nakagawa and T. Matsumoto.

References

Beaudet M, Messier C (2002) Variation in canopy openness and light transmission following selection cutting in northern hardwood stands: an assessment based on hemispherical photographs. Agric For Meteorol 110:217-228

Bruna EM (1999) Seed germination in rainforest fragments. Nature 402:139

CanopOn (2003) User's manual for CanopOn 2. Available from http://takenaka-akio.cool.ne.jp/etc/canopon2/
Cascante A, Quesada M, Lobo JJ, Fuchs EA (2002) Effects of dry tropical forest fragmentation on the reproductive success and genetic structure of the tree Samanea saman. Conserv Biol 16:137-147
Deka RN, Wairiu M, Mullins PWC, Mullins E, Veenendaal EM, Towsend J (1995) Use and accuracy of the filter-paper technique for measurement of soil matric potential. Eur J Soil Sci 46:233-238
Ferraz G, Russell GJ, Stouffer PC, Bierregaard RO Jr, Pimm SL, Lovejoy TE (2003) Rates of species loss from Amazonian forest fragments. Proc. Natl Acad Sci USA 100:14069-14073
Gascon C, Lovejoy TE, Bierregaard RO, Malcolm JR, Stouffer PC, Vasconcelos HL, Laurance WF, Zimmerman B, Tocher M, Borges S (1999) Matrix habitat and species richness in tropical forest remnants. Biol Conserv 91:223-229
Ichikawa M (2003) One hundred years of land-use changes: political, social, and economic influences on an Iban village in Bakong river basin, Sarawak, East Malaysia. In: Tuck-Po L, de Jong W, Ken-ichi A (eds) The political ecology of tropical forests in Southeast Asia: historical perspectives. Kyoto University Press and Trans Pacific Press, pp 177-199
Laurance WF, Delamônica P, Laurance SG, Vasconcelos HL, Lovejoy TE (2000) Rainforest fragmentation kills big trees. Nature 404:836
Laurance WF, Lovejoy TE, Vasconcelos HL, Bruna EM, Dedham RK, Stouffer PC, Gascon C, Bierregaard RO,
Laurance SG, Sampaio, E (2002) Ecosystem decay of Amazonian forest fragments: a 22-year investigation. Conserv Biol 16:605-618
Matthews E (2001) Understanding the FRA 2000. In: Forest Briefing 1, World Resources Institute, Washington, DC
Medellín RA, Equihua M (1998) Mammal species richness and habitat use in rainforest and abandoned agriculture fields in Chiapas, Mexico. J Appl Ecol 35:13-23
SAS Institute (2005) JMP user's guide. SAS Institute, Cary, NC, USA
Wilson EO (1989) Threats to biodiversity. Sci Am 261:108-116

Effects of Deforestation on Butterfly Diversity in and around Lambir Hills National Park, Sarawak, Malaysia

Takao Itioka[1], Taizo Tzuchiya[1], Daigo Matsumoto[1], Masaya Yago[2], Osamu Yata[3], Kuniyasu Momose[4], Tohru Nakashizuka[5]
[1]Graduate School of Human and Environmental Studies,
Kyoto University, Kyoto, 606-8501, Japan
[2]Graduate School of Science, University of Tokyo, Tokyo, 113-0033, Japan
[3]Graduate School of Social and Cultural Studies, Kyushu University, 810-8560, Japan
[4]Faculty of Agriculture, Ehime University, Matsuyama, 790-8566, Japan
[5]Graduate School of Life Sciences, Tohoku University, Sendai, 980-8578 Japan

Introduction

Tropical rainforests have been greatly disturbed by various kinds of human activities. In the lowlands of Sarawak, Malaysia, shifting cultivation based on slash-and-burn agriculture has been widely and conventionally adopted (Ichikawa 2003, 2004). The shifting cultivation accompanies deforestation of primary or secondary forests and is thus likely to influence the biodiversity of these forests. Moreover, this practice usually generates patches of remnant primary (undisturbed) forests and increasingly leads to isolation of these remnant areas (i.e., to forest fragmentation). This isolation is itself thought to reduce biodiversity in remnant forests (Laurence and Bierregaard 1997). To conserve the extremely rich biodiversity of the lowland dipterocarp forests of Sarawak, and to support the required policy development, the effects of human activities (e.g., shifting cultivation) on biodiversity must be qualitatively and quantitatively assessed.

From this perspective, various kinds of arthropods, which are considered to be the richest taxon in terms of species diversity, have been studied (e.g. Vasconcelos 1999, Willott 1999, Willott et al. 2000, Davis et al. 2001, Watt et al. 2002). Among these species, butterflies are a good indicator of biodiversity because they are mostly oligophagous herbivores. They are thus likely to reflect the status and species composition of the vegetation and the characteristics of microhabitats because they are easy to monitor by means of relatively brief, low-labor field surveys based on the observation of flying adults and because it is relatively easy to identify the different species.

In this study, we investigated the butterfly community in a lowland dipterocarp forest in Sarawak and in patchily distributed secondary forests around this primary forest. In particular, we determined the absence and presence of butterfly species in forest stands of different ages based on the elapsed time since slash-and-burn agriculture had been abandoned and in primary forest. By comparing the patterns of absence and presence among forest types, we attempted to determine the effects of the deforestation that accompanies shifting agriculture on the diversity of butterfly species.

Methods

All of our research was conducted in and around the Lambir Hills National Park (LHNP), Sarawak, Malaysia (4°2'–4°11'N, 113°50'–114°3'E; 150 to 200 m a. s. l.). The area of LHNP is ca. 70 km^2 (Yumoto and Nakashizuka 2005), and the climate is humid-tropical, with only a weak seasonal change in rainfall (Kato et al. 1995). Most of LHNP is covered by lowland mixed dipterocarp forest. Details of the vegetational features were described by Kato et al. (1995). The park is surrounded by secondary forest of different stand ages (i.e., different elapsed times after the last slash-and-burn cultivation) and by cultivated fields, rice paddies, extensively managed rubber plantations, and intensively managed oil palm plantations.

In the secondary forest around LHNP, we chose four stand types for our survey: sapling secondary forest stands (abbreviated as SSF) that were 1 year old at the initial time of the survey, young secondary forest stands (YSF) and extensively managed rubber plantations (ERP) that were 7 to 15 years old, and old secondary forest stands (OSF) that were more than 30 years old. In ERP, rubber trees were planted and extensively cultivated, but some of the dominant tree species observed in YSF were also present and grew to a size similar to that of their conspecifics in YSF. In addition, we studied two types of remnant primary forest that were isolated from the primary forest in the national park: distant remnant forest stands (DRF) and nearby forest stands (NRF). The former were more than 1.0 km from the reserved area and the latter were located within 500 m of the border of the reserved area.

For each of the stand types except OSF, we chose three representative study stands; for OSF, we chose six study stands. In each of the 21 forest stands, we established a 100-m line transect at the approximate center of the stand. We also randomly chose two open areas (forest gaps or bordering areas) 20 m in diameter in each forest stand. In addition to the six types of forest stands, we randomly set up three line transects, separated by more than 800 m, in the reserved primary forest of LHNP; we called this type of forest "primary forest" (PF). For each of the line transects in PF, we chose two open areas similar to those in the other six types of forest stand. Nakagawa et al. (2006) provided more details on stand characteristics such as tree species composition, total basal area of trees, and physical environmental conditions. Our study plots were generally similar to those in Nakagawa's study.

We conducted surveys to determine the absence and presence of butterfly species along the line transects and in the two open areas in each study stand. To perform our census in each study stand, we walked along the line transect for ca. 10 min and stayed in each of the open areas for ca. 10 min. During this time, we attempted to capture all butterflies that we encountered. More than half of the butterflies observed were caught using nets and then all caught were identified to the species level. We were also able to visually identify most butterflies that escaped our nets to the species level. We were unable to identify fewer than about 5% of the butterflies, and we omitted these individuals from the census data. All the species were recorded and some butterflies were mounted as voucher specimens, especially for individuals of species that are difficult to identify. The census at each site, which lasted for 30 to 45 min. in total, including handling time, took place in a period under clear skies between 0930 and 1300. The absence and presence data for each species and forest type was obtained twice during this period. We obtained four data matrices (presence or absence of a given species in each type of forest): in August 2003, September 2003, January 2005, and June 2005.

Based on these four matrices, we calculated the number of species observed in each stand. We then compared this number among forest types in order to determine the effects of deforestation on the butterfly species diversity.

Results and Discussion

We identified a total of 159 butterfly species throughout the study period. The number of species observed at least once for each type of forest stand is shown in Fig. 1. The mean number of species was highest in the primary forest stands (PF) and second highest in the nearby remnant forest stands (NRF), followed by the distant remnant forest stands (DRF), the old secondary forest stands (OSF), and the young secondary forest stands (NSF). It was lowest in the sapling secondary forest stand (SSF) and second lowest in the extensively managed rubber plantations (ERP), suggesting that both stand types have substantial negative effects on the butterfly diversity. Approximately 90% of all species recorded throughout the study period were observed in PF. These results suggest that deforestation due to slash-and-burn agriculture drastically decreased the butterfly species diversity in and around the affected area and that it is likely to take a long time (at least several decades based on the results for OSF) for butterfly species diversity to recover. In addition, forest fragmentation (NRF and DRF stands) also remarkably decreased the butterfly species diversity (to roughly half the levels in PF).

The results of our study clearly demonstrate that the disappearance of or large reductions in the area of primary forest clearly threatens to seriously damage the butterfly species diversity in lowland dipterocarp forests of the Southeast Asian tropics. It is likely that similar adverse consequences will occur for other species, but further research will be required to quantify these losses.

References

Davis AJ, Holloway JD, Huijbregts H, Krikken J, Kirk-Spriggs AH, Sutton SL (2001) Dung beetle as indicators of change in the forests of northern Borneo. J Appl Ecol 38:593-616

Ichikawa M (2003) Sarawaku shu Iban Sonraku no Setai nimirareru Seigyo Sentaku (Choice of livelihood activities by Iban household members in Sarawak, East Malaysia). Tropics 12:201-219 (in Japanese with English summary)

Ichikawa M (2004) Relationships among secondary forests and resource use and agriculture, as practiced by the Iban of Sarawak, East Malaysia. Tropics 13:269-286

Inoue T, Yumoto T, Hamid AA, Lee HS, Ogino K (1995) Construction of a canopy observation system in a tropical rainforest of Sarawak. Selbyana 16:24-35

Kato M, Inoue T, Hamid AA, Nagamitsu T, Merdek MB, Nona AR, Itino T, Yamane S, Yumoto T (1995) Seasonality and vertical structure of light-attracted insect communities in a dipterocarp forest in Sarawak. Res Popul Ecol 37:59-79

Laurance WF, Bierregaard RO Jr (eds) (1997) Tropical Forest Remnants: Ecology, Management, and Conservation of Fragmented Communities. The University of Chicago Press, Chicago.

Nakagawa M, Miguchi H, Nakashizuka T (2006) The effects of various forest uses on small mammal communities in Sarawak, Malaysia. For Ecol Manage 231:55-62

Vasconcelos HL (1999) Effects of forest disturbance on the structure of ground-foraging ant communities in central Amazonia. Biodiversity and Conservation, 8:409-420

Watt AD, Stork NE, Bolton B (2002) The diversity and abundance of ants in relation to forest disturbance and plantation establishment in southern Cameroon. J Appl Ecol 39:18-30

Willott SJ (1999) The effects of selective logging on the distribution of moths in a Bornean rainforest. Phil Trans R Soc Lond B, 354:1783-1790

Willott SJ, Lim DC, Compton SG, Sutton SL (2000) Effects of selective logging on the butterflies of a Bornean rainforest. Conservation Biology. 14:1055-1065

Yumoto T, Nakashizuka T (2005) The canopy biology program in Sarawak: scope, methods, and merit. In: Roubik DW, Sakai S, Hamid AA (eds) Pollination ecology and the rain forest Sarawak studies. Springer, New York, pp 13-21

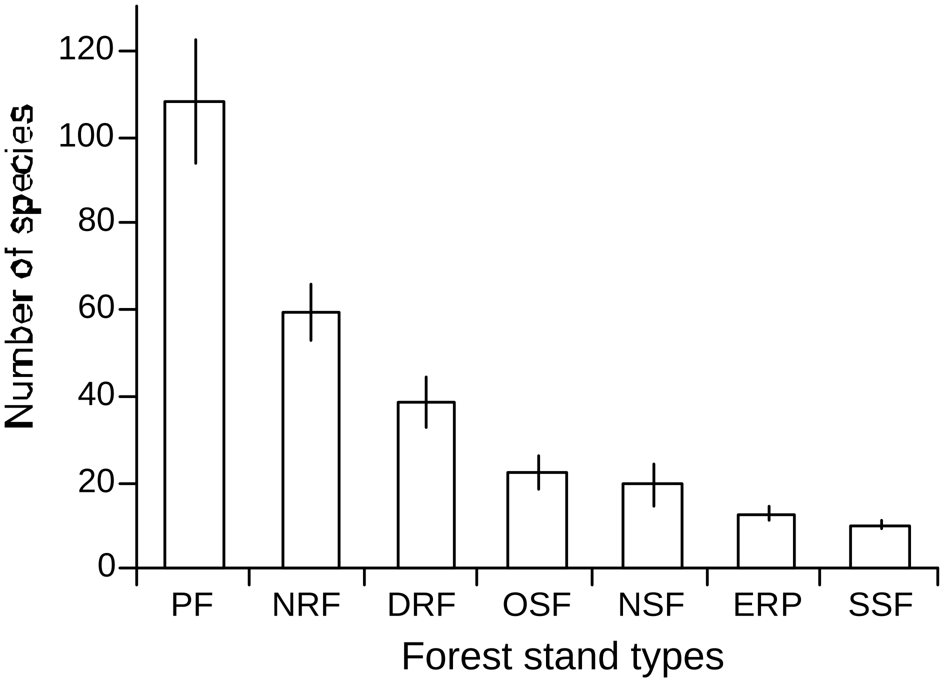


Figure 1. Number of species observed at least once for each forest stand types. Bars and vertical lines indicate the means and standard deviations, respectively. See text for abbreviations of the forest stand types.

Effects of Forest Changes after the Abandonment of Slash-and-Burn Cultivation on the Beetle Diversity in Sarawak, Malaysia

Keiko Kishimoto-Yamada [1*], Takao Itioka [1], Kuniyasu Momose [2], Tohru Nakashizuka [3]
[1] Takao Itioka Laboratory, Graduate School of Human and Environmental Studies, Kyoto University, Yoshida Nihonmatsu-cho, Sakyo-ku, Kyoto 606-8501, Japan
**E-mail: kky@mbox.kudpc.kyoto-u.ac.jp*
[2] Department of Forest Resources, Ehime University, Japan
[3] Graduate School of Life Sciences, Tohoku University, Japan

Introduction

Human impacts on the biodiversity of tropical forests are a widespread concern. To date, several studies have estimated these impacts by using various kinds of insect as indicators (e.g., Lawton et al. 1998; Liow et al. 2001). Such insect indicators have revealed a loss of species in secondary forests and cultivated fields compared to the original species diversity in tropical primary forests (e.g., Chey et al. 1997; Vasconcelos et al. 2000; Davis et al. 2001).

Slash-and-burn agriculture is an important and problematic human impact on forests, leading to significant changes. Around the primary forest of Sarawak, Malaysia, various kinds of forests have developed that represent different developmental stages after the abandonment of slash-and-burn cultivation. Microhabitat structures such as canopy openness, tree species richness, and tree density differ among these forests (Nakagawa et al. 2006). Have these forest changes affected on the insect diversity? Unfortunately, the effects of the different ages of the regenerated forests that resulted from the abandonment of slash-and-burn cultivation on insect diversity are poorly understood.

Since beetles exhibit extreme diversity in form and function, we chose the diversity of this taxon as a useful indicator of the effects of forest changes on biodiversity. In this study, we estimated the species richness, abundance, and composition of beetle assemblages in primary forests and in forests at different stages of regeneration after the abandonment of slash-and-burn cultivation.

Methods

Study Site

The study was conducted from August to September 2003 in and around Lambir Hills National Park, Sarawak, Malaysia (4º20'N, 113º50'E, 150 to 200 m a.s.l.). We established 12 study plots (100x20 m) in each of six forest types representing different stand ages: primary forest (plots H2 and H5), fragmented primeval forest (P2, P7), new fallow (1 year after abandonment; B1, B4), young fallow (5 to 6 years after abandonment; T1, T4), old fallow (>20 years after abandonment; F4, F5), and rubber plantation (G0, G9). Details of the study plots are provided by Nakagawa et al. (2006).

Insect Collections

The beetles were collected in the 12 study plots using a net with a 150-cm stretch at its longest extent. At each plot, we selected 100 thickets and swung a net 10 times in each thicket so that the sweeping covered ca. 196.25 m^2 foliage in total at each plot. The collected beetles were sorted to the family and morphospecies level on the basis of external characteristics.

Diversity and Similarity indices

Simpson's index of diversity (Lande 1996) was adopted to understand what degrees of the beetle diversity were at the 12 study plots. We also calculated Morishita's similarity index (C_λ) (Morishita 1959) to assess the similarity of the chrysomelid fauna between pairs of plots in all combinations of the 12 study plots.

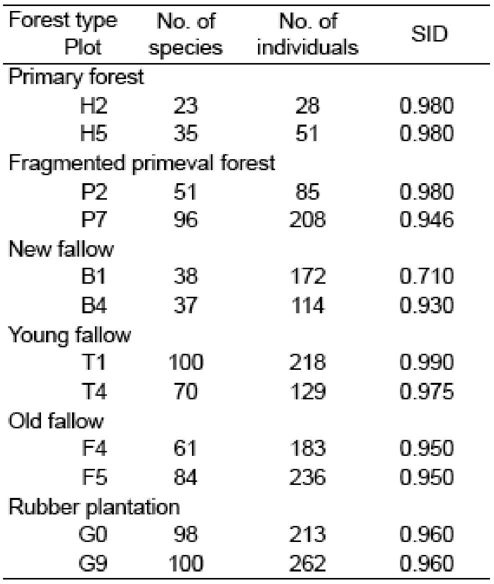
Results and Discussion

Beetle family composition, species richness, and abundance

Of the 166 coleopteran families listed by Lawrence and Newton (1995), 54 families were recorded in the 12 study plots. In terms of the number of species, Chrysomelidae was the most abundant family (ca. 23% of the total) caught throughout the study period, followed by Curculionidae (9%) and Anthicidae (7%). In terms of the number of individuals, Chrysomelidae was the most abundant (32%), followed by Anthicidae (16%) and Coccinellidae (7%). In Sabah, Malaysia, the most abundant coleopteran families were Staphylinidae and Pselaphidae (Chung et al. 2000). The differences in family composition may have resulted from the sampling methods; Chung et al. used three different methods to collect beetles, including ground-dwelling beetles. The most abundant families in the present study were the plant-associated beetles (Chrysomelidae and Curculionidae) and potential predators of herbivorous insects (Anthicidae and Coccinellidae).

The total number of individuals captured during our study was 1937, representing at least 556 species. The number of species was lowest in the primary forest and new fallow plots, but the abundances (number of individuals) shown in the new fallow plots were high (Table 1). The diversity indices were lower in the new fallow plots than in the other plots; in all plots except the new fallow plots, the diversity indices tended to be high (> 0.95; Table 1). A previous analysis at our study site using various microhabitat structure variables (Nakagawa et al. 2006) distinguished three groups of forest types: (A) primary forest plots, (B) new fallow plots, and (C) other plots. Since the plots for groups A and C were characterized by relatively high basal area, tree density, and tree species richness, the resulting microhabitat structures may enhance the beetle diversity in these plots. The primary forest plots were relatively shady, with a thick canopy layer, and we never included any of the gaps that occasionally appeared in primary forests in our study. Thus, we may

Table 1 The number of species, abundances (number of individuals), and Simpson's index of diversity (SID) of beetles in the 12 study plots



Forest type Plot	No. of species	No. of individuals	SID
Primary forest			
H2	23	28	0.980
H5	35	51	0.980
Fragmented primeval forest			
P2	51	85	0.980
P7	96	208	0.946
New fallow			
B1	38	172	0.710
B4	37	114	0.930
Young fallow			
T1	100	218	0.990
T4	70	129	0.975
Old fallow			
F4	61	183	0.950
F5	84	236	0.950
Rubber plantation			
G0	98	213	0.960
G9	100	262	0.960

have underestimated the species richness on the primary forest floor, because shady floor and brighter gaps are often mixed on the primary forest floor.

Species richness and abundance (number of individuals) data revealed that Chrysomelidae dominated most of the study plots (Table 2). Carabidae, most of which are carnivores, were abundant in one primary forest plot, but their numbers were low in other plots.

Table 2 List of top two abundant families in regard of species number and abundances in the 12 study plots

Top two abundant families in regard of species richness						
	H2	H5	P2	P7	B1	B4
1	Curculionidae (36%)	Chrysomelidae (23%)	Chrysomelidae (24%)	Chrysomelidae (19%)	Chrysomelidae (32%)	Chrysomelidae (34%)
2	Elateridae (14%) Chrysomelidae (14%)	Carabidae (11%)	Anthicidae (20%)	Anthicidae (18%)	Anthicidae (13%)	Coccinellidae (10%) Elateridae (10%)
	T1	T4	F4	F5	G0	G9
1	Chrysomelidae (28%)	Chrysomelidae (27%)	Chrysomelidae (38%)	Chrysomelidae (30%)	Chrysomelidae (14%)	Chrysomelidae (22%)
2	Anthicidae (7%) Curculionidae (7%)	Coccinellidae (11%)	Anthicidae (11%)	Anthicidae (10%)	Anthriidae (10%) Curculionidae (10%)	Coccinellidae (11%)
Top two abundant families in regard of abundances						
	H2	H5	P2	P7	B1	B4
1	Curculionidae (38%)	Carabidae (22%)	Chrysomelidae (25%)	Anthicidae (23%)	Anthicidae (59%)	Chrysomelidae (49%)
2	Chrysomelidae (10%) Coccinellidae (10%) Elateridae (10%)	Chrysomelidae (20%)	Anthicidae (22%)	Ptinidae (20%)	Chrysomelidae (27%)	Anthicidae (12%)
	T1	T4	F4	F5	G0	G9
1	Chrysomelidae (23%)	Chrysomelidae (40%)	Chrysomelidae (42%)	Chrysomelidae (50%)	Chrysomelidae (30%)	Chrysomelidae (30%)
2	Anthicidae (16%)	Phalacridae (9%)	Cantharidae (16%)	Cantharidae (9%)	Anthicidae (12%)	Cantharidae (15%)

Numbers in the parentheses represent the propotion of the family to total species or individuals captured in each plot

Distribution of species-abundance in the 12 study plots

The species rank-abundance curves showed that the primary forest plots and one of the fragmented primeval forest plots had relatively shallow curves compared to those of the other plots (Fig. 1). This suggests that the evenness of the species abundance in the former plots is high. A single species of Anthicidae dominated (more than half of all individuals) in one new fallow plot (B1; Fig. 1). Such dominant species may be rare in primary forests and forests that retain most of the characteristics of primary forests.

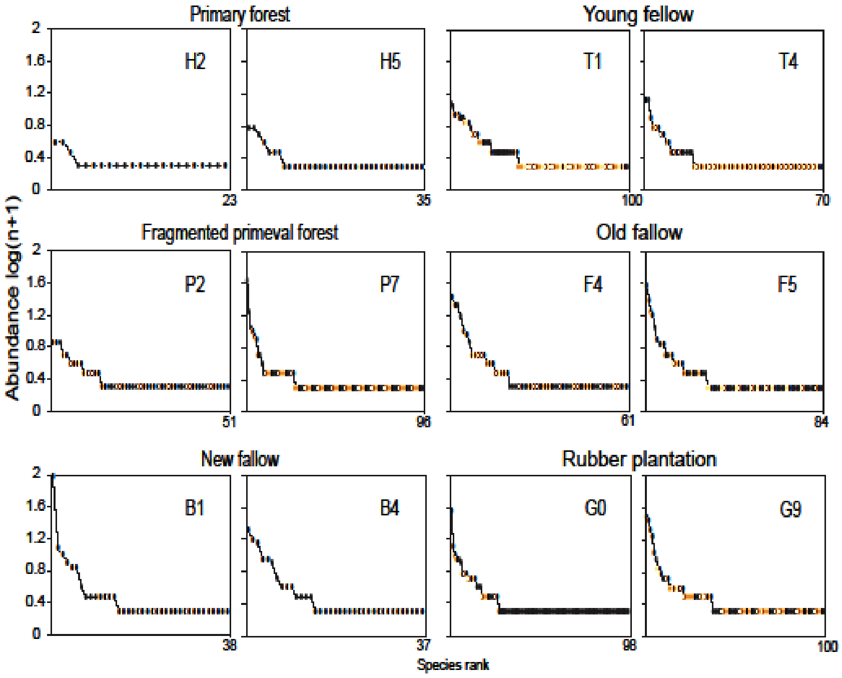


Fig. 1 Species rank-abundance curves for the beetles at the 12 study plots

Chrysomelid fauna in the 12 study plots

For the paired combinations of the same forest type, we found high similarity indices for the chrysomelid fauna in primary forest, new fallow, and old fallow, but the similarity indices were low between the two plots belonging to the fragmented primeval forest, young fallow, and rubber plantation (Table 3). The highest similarity index (0.990) was between H5 and G9 (Table 3), suggesting that the chrysomelid faunas in these plots strongly resemble each other; however, the microhabitat structures of these two plots were not particularly similar (Nakagawa et al. 2006). The chrysomelid species found in the new fallow plots rarely appeared in other plots and never appeared in primary forest and fragmented primeval forest (Fig. 2). Only two species caught in some plots (P7, T1, F4, G0, G9) were found in the primary forest plots (Fig. 2), suggesting that few species that inhabit deforested areas can invade primary forests.

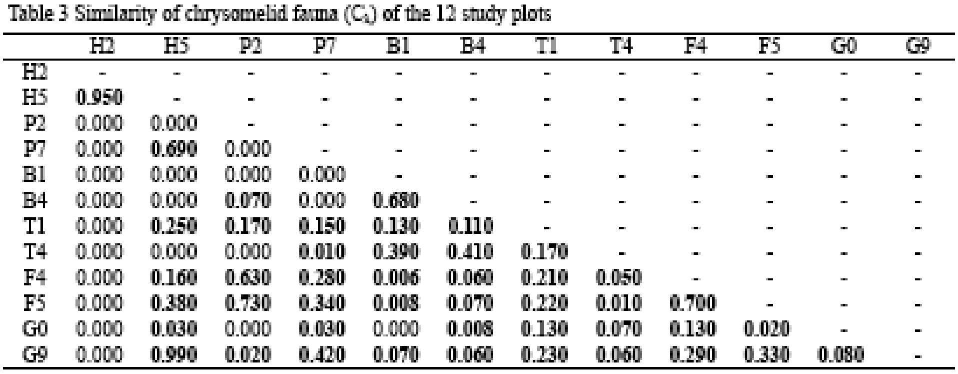


Table 3 Similarity of chrysomelid fauna (C_λ) of the 12 study plots

	H2	H5	P2	P7	B1	B4	T1	T4	F4	F5	G0	G9
H2	-	-	-	-	-	-	-	-	-	-	-	-
H5	**0.950**	-	-	-	-	-	-	-	-	-	-	-
P2	0.000	0.000	-	-	-	-	-	-	-	-	-	-
P7	0.000	**0.690**	0.000	-	-	-	-	-	-	-	-	-
B1	0.000	0.000	0.000	0.000	-	-	-	-	-	-	-	-
B4	0.000	0.000	**0.070**	0.000	**0.680**	-	-	-	-	-	-	-
T1	0.000	**0.250**	**0.170**	**0.150**	**0.130**	**0.110**	-	-	-	-	-	-
T4	0.000	0.000	0.000	**0.010**	**0.390**	**0.410**	**0.170**	-	-	-	-	-
F4	0.000	**0.160**	**0.630**	**0.280**	**0.006**	**0.060**	**0.210**	**0.050**	-	-	-	-
F5	0.000	**0.380**	**0.730**	**0.340**	**0.008**	**0.070**	**0.220**	**0.010**	**0.700**	-	-	-
G0	0.000	**0.030**	0.000	**0.030**	0.000	**0.008**	**0.130**	**0.070**	**0.130**	**0.020**	-	-
G9	0.000	**0.990**	**0.020**	**0.420**	**0.070**	**0.060**	**0.230**	**0.060**	**0.290**	**0.330**	**0.080**	-

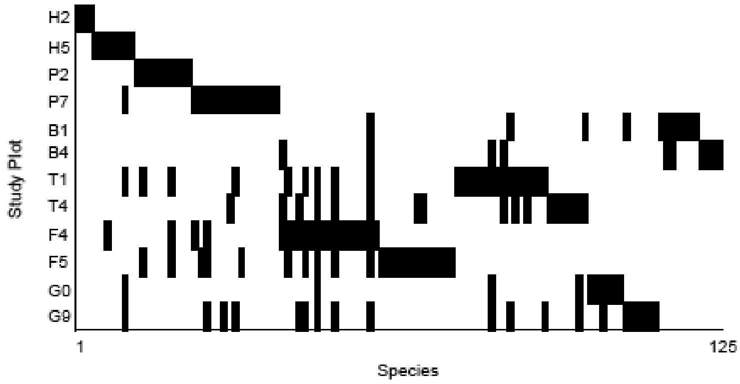


Fig. 2 Species overlap of chrysomelid assemblages among the 12 study plots

Acknowledgments

We are grateful to the Forest Department of Sarawak and the Sarawak Forestry Corporation for their permission to conduct this study and for their assistance with our fieldwork. This study was financially supported by RIHN research project 2–2.

References

Chey VK, Holloway JD, Speight MR (1997) Diversity of moths in forest plantations and natural forests in Sabah. Bull Entomol Res 87:371–385

Chung AYC, Eggleton P, Speight MR, Hammond PM, Chey VK (2000) The diversity of beetle assemblages in different habitat types in Sabah, Malaysia. Bull Entomol Res 90:475–496

Davis A J, Holloway JD, Huijbregts H, Krikken J, Kirk-Spriggs AH, Sutton SL (2001) Dung beetles as indicators of change in the forests of northern Borneo. J Anim Ecol 38:593–616

Lande R (1996) Statistics and partitioning of species diversity, and similarity among multiple communities. Oikos 76:5–13

Lawrence JF, Newton AF Jr (1995) Families and subfamilies of Coleoptera (with selected genera, notes, references and data on family-group names). In: Pakaluk J, Slipinski SA (eds) Biology, phylogeny, and classification of Coleoptera papers celebrating the 80th Birthday of Roy A. Crowson. Muzeum i Instytut Zoologii PAN, Warszawa, pp 779–1006

Lawton JH, Bignell DE, Bolton B, Bloemers GF, Eggleton P, Hammond PM, Hodda M, Holt RD, Srivastava DS, Watt AD (1998) Biodiversity inventories, indicator taxa and effects of habitat modification in tropical forest. Nature 391:72–76

Liow LH, Sodhi N, Elmqvist T (2001) Bee diversity along a disturbance gradient in tropical lowland forests of south-east Asia. J Appl Ecol 38: 80–192

Morishita M (1959) Measuring of interspecific association and similarity between communities. Mem Fac Sci Kyushu Univ Ser E (Biol) 3:65–80

Nakagawa M, Miguchi H, Nakashizuka T (2006) The effects of various forest uses on small mammal communities in Sarawak, Malaysia. For Ecol Manage 231:55–62

Vasconcelos HL, Vilhena JMS, Caliri GJA (2000) Responses of ants to selective logging of a central Amazonian forest. J Appl Ecol 37:508–514

Effects of Traditional Forest Uses on the Number of Colonies of Army Ants

Takashi Matsumoto[1], Takao Itioka[1], Seiki Yamane[2]
[1]*Graduate School of Human and Environmental studies, Kyoto University*
[2]*Earth and Environmental Sciences, Faculty of Science, Kagoshima University*

Introduction

The rapid and widespread loss of biological diversity is a matter of considerable concern. Destruction of tropical rain forests, one of the most diverse terrestrial ecosystems, is one of the major factors responsible for the high rates of species extinction in recent years (Myers 1988). Globally, tropical rain forests are disappearing at a rate of 12 million ha per year (Cyranoski 2007). Furthermore, less than 5% of the total land area of tropical rainforests is protected as reserves or national parks (Western 1989). Thus, the development of methods of using tropical rain forests that can prevent biodiversity loss is urgently needed.

In Southeast Asia, swidden (slash-and-burn) agriculture has been widely used by local residents and is frequently regarded as one of the primary causes of forest degradation and loss (Freeman 1955; Lanly 1982). Others, however, have stated that traditional methods of swidden agriculture are sustainable and maintain the ecosystem because of long fallow periods as well as the complex mosaic pattern of agricultural fields, abandoned fields in various stages of forest regrowth, and primary forest (Salafsky 1993; Aumeerudy and Sanonnens 1994; Coomes et al. 2000). To better understand whether traditional swidden agriculture is sustainable in terms of conservation of biodiversity, we need to examine whether biodiversity in secondary forests recovers to initial levels following cultivation and, if so, how long it takes.

In the present study, we compared encounter rates between primary forests and secondary forests with various fallow periods for the top predator found among litter arthropods, the army ant (*Aenictus* and *Leptogenys* species). The top predator was chosen as an indicator for biodiversity loss caused by disturbance because it is vulnerable to disturbance and degradation (Noss et al. 1996) and also because it frequently plays an important role in structuring communities (Polis et al. 2000; Schmitz et al. 2000; Schmitz and Suttle 2001).

Material and Methods

Study site

The study was conducted in and around Lambir Hills National Park, Sarawak, Malaysia (4°20'N, 113°50'E; 150 to 200 m a.s.l.). Details of the park were described in Inoue et al. (1995). Local residents near the park have practiced swidden agriculture to cultivate rice and vegetables for about 100 years (Ichikawa 2002). To examine the effect of traditional swidden agriculture on biodiversity, we established two plots within the park, 11 plots in secondary forests at various developmental stages, and six plots in isolated primary forests near the park. Secondary forests were categorized into three groups depending on time since the abandonment of cultivation: new fallow (NF), <2 years after abandonment (N=3); young secondary forest (NSF), 5 years after abandonment (N=3); and old secondary forest (OSF), >20 years after abandonment

(*N*=5).

Field census

We visually located army ant colonies from August to September 2003 and from January to February in 2005. Above-ground ant foraging activity is readily detected by observers because the ants move in coordinated columns on the forest floor, tree trunks, or vines. Three 1-h searches were conducted at each plot during each census period. Censuses were performed between 0800 and 1700 hours on days with good weather. Results from the three 1-h searches were combined for analysis for each census period. Prior to analysis, the numbers of ant colonies were log-transformed ($y = \log [x + 0.5]$) to stabilize the variances (Yamamura 1999).

Results

A total of nine *Aenictus* species was observed in 26 colonies. *Aenictus laeviceps* morphotypes L1 and L2 were the dominant species (53% of the total colonies in 2003 and 55% in 2005). *Aenictus inflatus*, *A. camposi*, *A. hottai*, and *A. cornutus* were not detected in the primary forests, and *A. gracilis* was observed only in the isolated primary forest (Table 1a). All of the *Aenictus* colonies detected in this study were on raid and no emigrating colony was observed. The number of *Aenictus* colonies differed significantly among forest types (repeated ANOVA: $F = 6.42$, $df = 4$, $P = 0.004$; Fig. 1a), with the highest number of colonies found in the primary forest, an intermediate number found in young and old secondary forests, and the lowest number in new fallow (Tukey-Kramer HSD: $P < 0.05$; Fig 1a). No significant differences were found between years (repeated ANOVA: $F = 0.006$, $df = 1$, $P = 0.93$) or for the interaction between year and forest type (repeated ANOVA: $F = 2.08$, $df = 4$, $P = 0.13$; Fig 1a).

For *Leptogenys*, we detected six species (36 colonies) in 2003 and four species (22 colonies) in 2005 (Table 1b). In contrast with *Aenictus*, differences in encounter rates with *Leptogenys* were not significant among forest types, although there was a weak tendency for more *Leptogenys* to be encountered in primary forests (repeated ANOVA: $F = 2.43$, $df = 4, 14$, $P = 0.10$; Fig 1b). No significant differences were found between years or for the interaction between year and forest type ($F = 2.82$, $df = 1, 1$, $P = 0.12$ for year; $F = 0.25$, $df = 4, 14$, $P = 0.90$ for interaction; Fig 1b).

Discussion

When *Aenictus* was used as a bioindicator, traditional swidden agriculture was shown to have a serious impact on biodiversity in the tropical rain forest of Sarawak. Once an area was farmed using swidden agriculture, the effect of the disturbance lasted more than 20 years, although some Dipterocarp trees recovered at old secondary forests (Momose et al., unpublished data). Vasconcelos (1999) suggested that ground-dwelling ant fauna recovered in as few as 25 years when the intensity of the disturbance was low, so it seems that the impact of traditional swidden agriculture on biodiversity is more severe than was previously expected. It is possible that a longer fallow period would increase the abundance of *Aenictus*. However, no *Aenictus* was detected in old secondary forests (>100 years after abandonment). Thus, it seems to be unrealistic to expect that biodiversity will be maintained by extending the fallow period.

Some *Aenictus* colonies were observed in young and old secondary forests, although in smaller numbers than in the primary forest. All of the secondary forests in which army ants were observed, except one, were located proximate to continuous primary forest. This means that remnant primary forest may have contributed to the maintenance of biodiversity in the surrounding area. In terms of managing forest usage to sustain biological diversity, our study suggested that preserving enough primary forest would be more effective than extending the fallow period after traditional swidden agriculture.

Four *Aenictus* species (*A. inflatus, A. camposi, A. hottai,* and *A. cornutus*) were observed only in secondary forests. All four species have previously been detected in a continuous primary forest (Yamane and Hashimoto 1999; Yamane, unpublished data). Therefore, it is unlikely that segregation of the *Aenictus* group occurred between forest types.

In contrast to *Aenicitus*, the number of colonies of *Leptogenys* did not differ among forest types. *Aenictus* preys exclusively on immature social insects (Gotward 1976), whereas the diet of *Leptogenys* is much broader (Maschwitz and Mühlenberg 1975; Suzzoni et al. 2000; but see Mashwitz and Schönegge 1983). Using *Eciton burchelli* and *Labidus praedator,* who prey on almost all kinds of arthropods (Rettenmeyer 1963a,b), Roberts et al. (2000) showed that the number of colonies of army ants did not differ significantly between a primary forest and a traditional shady coffee plantation. Future research is required about the relationship between the breadth of diet and the strength of tolerance to artificial disturbance among army ant groups.

Table 1a. List of *Aenictus* species encountered during the census in each forest type in 2003 (upper) and 2005 (lower)

2003		*Aenictus*								
Forest type	total	*A. laeviceps morpthotype L1*	*A. laeviceps morpthotype L2*	*A. gracilis*	*A. dentatus*	*A. inflatus*	*A. camposi*	*A. hottai*	*A. sp5 of sky*	*A. cornutus*
NF (n=3)	0	0	0	0	0	0	0	0	0	0
YSF (n=3)	0	0	0	0	0	0	0	0	0	0
OSF (n=5)	1	0	0	0	0	1	0	0	0	0
IPF (n=6)	9	3	2	2	1	0	0	0	0	1
CPF (n=2)	5	2	1	0	1	0	0	0	1	0

2005		*Aenictus*								
Forest type	total	*A. laeviceps morpthotype L1*	*A. laeviceps morpthotype L2*	*A. gracilis*	*A. dentatus*	*A. inflatus*	*A. camposi*	*A. hottai*	*A. sp5 of sky*	*A. cornutus*
NF (n=3)	0	0	0	0	0	0	0	0	0	0
YSF (n=3)	2	0	0	0	0	1	1	0	0	0
OSF (n=5)	2	1	0	0	1	0	0	0	0	0
IPF (n=6)	4	1	1	0	0	1	0	1	0	0
CPF (n=2)	3	2	1	0	0	0	0	0	0	0

NF, YSF, OSF, IPF, CPF designated new fallow, young secondary forest, old secondary forest, isolated primary forest, and continuous primary forest, respectively. See M&M for the definition of each forest type.

Table 1b. List of *Leptogenys* species encountered during the census in each forest type in 2003 (upper) and 2005 (lower)

2003	*Leptogenys*						
Forest type	total	*L. processionalis*	*L. diminuta*	*L. sp39 of sky*	*L. sp 6 of sky*	*L. mutabilis*	*L. chalybaea*
NF (n=3)	5	4	1	0	0	0	0
YSF (n=3)	1	0	0	1	0	0	0
OSF (n=5)	10	5	1	4	0	0	0
IPF (n=6)	11	2	5	3	1	0	0
CPF (n=2)	9	3	3	1	0	1	1

2005	*Leptogenys*						
Forest type	total	*L. processionalis*	*L. diminuta*	*L. sp39 of sky*	*L. sp 6 of sky*	*L. mutabilis*	*L. chalybaea*
NF (n=3)	2	2	0	0	0	0	0
YSF (n=3)	1	0	1	0	0	0	0
OSF (n=5)	5	2	2	1	0	0	0
IPF (n=6)	7	2	3	2	0	0	0
CPF (n=2)	7	3	2	1	0	0	1

NF, YSF, OSF, IPF, CPF designated new fallow, young secondary forest, old secondary forest, isolated primary forest, and continuous primary forest, respectively.
See M&M for the definition of each forest type.

Acknowledgments

We thank L. Chong (Sarawak Forestry Corporation), Dr. J. Kendawang (Forest Department Sarawak), and the staff of Lambir Hills National Park for allowing us to perform our study. We also thank H. Kaliang (Sarawak Forestry Corporation) for helping us take specimens from Sarawak for identification and Deline for mounting the ant specimens. This study was financially supported by RIHN research project 2-2 and a JSPS Research Fellowship for Young Scientists to T. M.

References

Aummeeruddy Y, Sansonnens B (1994) Shifting from simple to complex agroforestry systems: an example for buffer zone management from Kerinci. Agrofor Ayst 28:113–141

Coomes OT, Grimard F, Burt G (2000) Tropical forests and shifting cultivation: secondary forest fallow dynamics among traditional formers of the Peruvian Amazon. Ecol Econ 32:109–124

Cyranoski D (2007) Logging: the new conservation. Nature 446:608–610

Freeman JD (1955) Iban agriculture: a report on the shifting cultivation of hill rice by the Iban of Sarawak. HMSO, London

Gotward WHJr (1976) Behavioral observations on African army ants of the genus *Aenictus* (Hymenoptera: Formicidae). Biotropica 8:59–65

Ichikawa M (2002) Studies on uses of ecological resources at an Iban village in Bakong river basin, Sarawak. Ph D Thesis Kyoto University (in Japanese)

Inoue T, Yumoto T, Hamid AA, Seng LH, Ogino K (1995). Construction of a canopy observation system in a tropical rainforest of Sarawak. Selbyana 16:24–35.

Lanly JP (1982) Tropical forest resources. FAO Forestry Paper 30, FAO, Rome

Maschwitz U, Mühlenberg M (1975) Zur Jagdstrategie einiger orientalischer *Leptogenys-Arten* (Formicidae: Ponerinae). Oeocologia 20:65–83 (in German with an English summary)

Maschwitz U, Schönegge P (1983) Forage communication, nest moving recruitment, and prey specialization in the oriental ponerine *Leptogenys chinensis*. Oecologia 57:175–182

Myers N (1988) Tropical deforestation and climatic-change. Environ Conserv 15:293–298

Noss RF, Quigley HB, Hornocker MG, Merrill T, Paquet PC (1996) Conservation biology and carnivore conservation in the Rocky Mountains. Conserv Biol 10:949–963

Polis GA, Sears ALW, Huxel GR, Strong DR, Maron J (2000) When is a trophic cascade a trophic cascade? Tr Ecol Evol 15:473–475

Rettenmeyer CW (1963a) The behavior of Thysanura found with army ants. Ann Entomol Soc Am 56:170–174

Rettenmeyer CW (1963b) Behavioral studies of army ants. Univ Kan Sci Bull 44:281–465

Roberts DL, Robert JC, Petit LJ (2000) Use of premontane moist forest and shade coffee agroecosystems by army ants in Western Panama. Conserv Biol 14:192–199

Salafsky N (1993) Mammalian use of a buffer zone agroforestry system bordering Gunung Palung National Park, West Kalimantan, Indonesia. Conserv Biol 7:928–933

Schmitz OJ, Hamback PA, Beckerman AP (2000) Trophic cascades in terrestrial systems: A review of the effects of carnivore removals on plants. Am Nat 155:141–153

Schmitz OJ, Suttle KB (2001) Effects of top predator species on direct and indirect interactions in a food web. Ecology 82:2072–2081

Suzzoni JP, Schatz B, Dejean A (2000) Essential and alternative prey in a ponerine ant: variations according to the colony cycle. Life Sciences 32:1003–1008

Vasconcelos HL (1999) Effects of forest disturbance on the structure of ground-foraging ant communities in central Amazonia. Biodivers Conserv 8:407–418

Western D (1989) Conservation without parks: wildlife in a rural landscape. In: Western D, Pearl MC (eds) Conservation for the twenty-first century. Oxford University Press, New York, pp 158–165

Yamane S, Hashimoto Y (1999) A remarkable new species of the army ant genus *Aenictus* (Hymenoptera, Formicidae) with a polymorphic worker caste. Tropics 8:427–432

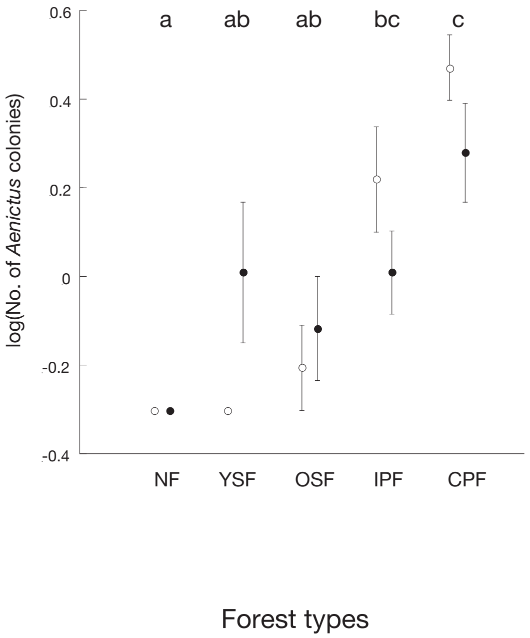


Figure 1a. Mean (±S.E.) of log-transformed encounter rates with *Aenictus* species within each forest type in 2003 (open circle) and 2005 (solid circle). Different letters among circle designated significant difference among forest types. NF, YSF, OSF, IPF, CPF designated new fallow, young secondary forest, old secondary forest, isolated primary forest, and continuous primary forest, respectively. See M&M for the definition of each forest type.

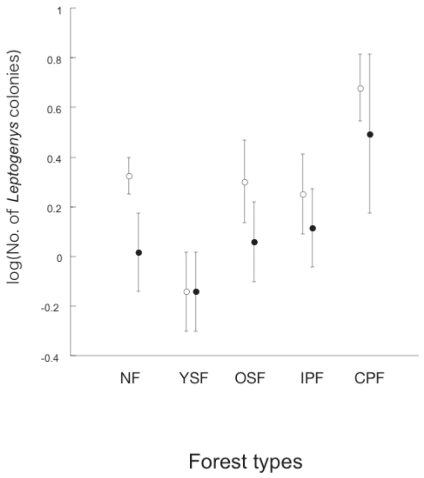


Figure 1b. Mean (±S.E.) of log-transformed encounter rates with *Leptogenys* species within each forest type in 2003 (open circle) and 2005 (solid circle). NF, YSF, OSF, IPF, CPF designated new fallow, young secondary forest, old secondary forest, isolated primary forest, and continuous primary forest, respectively. See M&M for the definition of each forest type.

Effects of Land-Use Changes on Bat Diversity in and around Lambir Hills National Park, Sarawak, Malaysia

Daisuke Fukuda[1], Oswald Braken Tisen[2], Kuniyasu Momose[3], Shoko Sakai[1]
[1]*Center for Ecological Research, Kyoto University, Hirano 509-3,Otsu, 520-2113, Japan*
[2]*Sarawak Forestry Corporation, Level 12, Office Tower, Hock Lee Center,*
Jalan Datuk Abang Abdul Rahim, 93450 Kuching, Sarawak, Malaysia
[3]*Faculty of Agriculture, Ehime University, 3-5-7, Tarumi, Matsuyama, 790-8566, Japan*

Introduction

Tropical forest landscapes are changing rapidly because of human activities. Approximately half of the potential tropical closed-canopy forest has already been removed and converted to other uses (Wright 2005). The effects of these anthropogenic changes on mammals have been studied across several taxonomic groups. Among these groups, bats (Chiroptera) are thought to be one of the most vulnerable taxa. Major threats to bat populations include the loss or reduction in quality of foraging habitat and habitat fragmentation (Racey and Entwistle 2002).

The order Chiroptera is divided into two suborders: the Megachiroptera, with a single family (Pteropodidae, ca. 163 species) and the Microchiroptera, with 17 families (ca. 814 species in total; Corbet and Hill 1992). Megachiropterans are known as Old World fruit bats and are distributed across Africa, tropical Asia, India, Australia, and their surrounding oceanic islands. Megachiropterans are relatively large (20 - 1500 g) and feed exclusively on plants (fruits, nectar, pollen, flowers, and leaves). In contrast, microchiropterans are found on every continent except Antarctica, are relatively small (1.5 to 150 g), and exhibit more diverse feeding habits (e.g., insectivorous, frugivorous, nectarivorous, ichthyophagous, and sanguivorous; Altringham 1996).

Recent studies have indicated that many bat species play important roles in tropical rain forests. Pollination by bats is a phenomenon restricted to the tropics and subtropics. Megachiropterans visit at least 141 plant species, including a number of commercially important plants (e.g., *Durio, Ceiba,* and *Parkia*) for nectar or pollen (Fujita and Tuttle 1991). Megachiropterans feed upon 145 genera of fruits and presumably disperse the seeds of the majority of the fruits they consume (Marshall 1985). In addition, approximately 70% of extant bat species are insectivorous and prey on a diverse range of insects (e.g., Lepidoptera, Diptera, Coleoptera, and Hemiptera; Jones and Rydell 2002).

Southeast Asia has the highest deforestation rate of any major tropical region, and currently, more than 50% of the land area in Asia is used for agricultural purposes (Zhao et al. 2006). The conversion of forests to cash-crop plantations (e.g., oil palm, rubber, and cocoa) is thought to be one of the major causes of the current high deforestation rates in the region (Primack and Corlett 2005). Such anthropogenic changes can create mosaics of fragmented vegetation, thereby greatly affecting the diversity, abundance, and feeding behavior of bats. Megachiropterans that inhabit these mosaic landscapes are expected to feed on crops because some agricultural plants may serve as food sources, and the bats can fly long distances from mosaic to mosaic. However, little is known about the effects and extent of the impacts of these anthropogenic changes.

The area surrounding a primary forest of the Lambir Hills National Park in Borneo is a typical example of such a mosaic landscape, including various agricultural lands and fragmented primary and secondary forests. In this study, we investigated the bat community in the mosaic landscape. Specifically, we explored differences in the density and diversity of megachiropterans between primary forests and agricultural lands within the landscape.

Methods

We conducted our research in and around the Lambir Hills National Park (LHNP), Sarawak, Malaysia (Fig. 1; 4°2'N, 113°50'E; ca. 150 m a.s.l.). One characteristic of the site was the high heterogeneity of vegetation. We selected four types of vegetation for bat censuses: primary forests, secondary forests, orchards, and oil palm plantations. The primary forests were intact lowland mixed dipterocarp forests within the LHNP. The park covers an area of 6949 ha and the height of emergent trees sometimes exceeds 70 m. Shanahan and Debski (2002) recorded 10 species of bats (five megachiropterans and five microchiropterans) in the park. The secondary forests were young forests that developed after slash-and-burn agriculture had been conducted by Iban villagers. Census points were established in three forests of varying ages: a 7-year-old forest dominated by *Vitex pinnata*, an approximately 30-year-old forest dominated by *Artocarpus elasticus*, and a >60-year-old forest dominated by A. *elasticus* (Nakagawa et al. 2006). Forest height varied among census points (2 - 25 m). These forests were surrounded by ponds, paddy fields, isolated intact forests, and rubber (*Hevea brasiliensis*) forests. The orchards were small (<5 ha) areas with many cultivated plants (e.g., *Durio kutejensis*, *Nephelium lappaceum*, *Carica papaya*, *Cocos* sp., *Musa* sp., *Parkia* sp., *Artocarpus integer*, *Lansium domesticum*, *Piper* sp., and *Saccharum* sp.) established by Iban villagers. Tree height ranged from 1 to 10 m. The orchards were located near villagers’ houses and were surrounded by ponds, paddy fields, rubber forests, and bamboo groves. The oil palm plantations were large-scale (ca. 4000 ha) continuous plantations of mature African oil palm (*Elaeis guineensis*) managed either by a corporation or by Iban villagers. Vegetation consisted of a complete monoculture, and the heights of oil palms were 10 to 20 m. The plantations shared borders with the primary and secondary forests.

Censuses were conducted four times between April 2005 and August 2006. We set mist nets on the ground along trails in the forests or forest edges. In the primary forests, we also set nets on canopy walkways (at a height of 15 to 35 m) for five nights. There were no significant differences between data from the ground and the walkways so the data were combined. We used two to four nets (24- to 36-mm mesh; 6 to 12 m long; 6.0 m high; eight shelves) per night and occasionally harp traps (The Austbat Harptrap, Faunatech and Austbat, Australia; 4.2 m2 in area; four nights in the primary forests and two nights in the orchards). Traps were set at sunset and checked at 15-min intervals. Sampling continued until 2300 hours unless it rained, since bat activities are usually very low in the rain. We recorded age, sex, morphological measurements, and reproductive state of the captured bats. Age class was determined by the degree of fusion of the epiphyseal plates on the phalanges, which can be determined without harming the bats (Kunz 1988). Bats with unfused epiphyseal plates were regarded as juveniles. The number of census points and sampling effort varied among vegetation type: 14 179 m2h (area of traps [m2] × sampling time [h]) in the primary forests (six census points), 8707 m2h in the secondary forests (three census points), 6526 m2h in the orchards (three census points), and 3382 m2h in the oil palm plantations (five census points).

We identified bat species according to Payne and Francis (1998). Because some studies have suggested the presence of two cryptic species within *Cynopterus brachyotis* that differ significantly in genetic and morphological characteristics as well as habitat preferences (Francis 1990; Abdullah et al. 2000; Abdullah 2003), we divided the species into two categories (C. *brachyotis* I and C. *brachyotis* II). We classified individuals of C. *brachyotis* based on their forearm length: in adult C. *brachyotis* I (the larger form), the forearm length ranged from 60 to 66 mm, whereas for adult C. *brachyotis* II (the smaller form), the forearm length was approximately 55 mm but always less than 60 mm (M.T. Abdullah, UNIMAS, pers. comm.). If individuals of the species were juveniles and their forearm lengths were less than 60 mm, they were recorded as "C. *brachyotis* (unidentifiable)".

A chi-squared test (Sokal and Rohlf 1973) was used to compare the number of captures of megachiropterans, microchiropterans, and each individual species among the vegetation types. In the chi-squared tests, the observed and expected numbers of captures were compared. The expected number of captures was calculated based on the assumption that the capture rate was equal for each vegetation type.

Simpson's index of diversity (Simpson 1949) was calculated for each vegetation type. Unidentifiable individuals of C. *brachyotis* (see above) were assigned to C. *brachyotis* I and C. *brachyotis* II based on the proportion of the two forms in the community.

Results

The total sampling effort (32 795 m2h) resulted in the capture of 495 bats representing 28 species in five families. The capture rate of megachiropterans differed significantly ($P < 0.001$) among the four vegetation types and was particularly high in the oil palm plantations and orchards compared to the primary and secondary forests (Table 1). The capture rate of microchiropterans also differed significantly ($P < 0.001$) among vegetation types and was lower in the oil palm plantations compared to the other three plant communities (Table 1).

For the eight bat species with relatively large sample sizes (>20 individuals), capture rate varied among vegetation types (Table 1). The capture rates of *Balionycteris maculata*, *Penthetor lucasii*, and *Hipposideros cervinus* were the highest in primary forests. Capture rates were lower in secondary forests than in primary forests for all species except *C. brachyotis* I and II. In the orchards, the capture rates of *Eonycteris spelaea*, *Macroglossus minimus*, and *Glischropus tylopus* were very high compared to those in the other three vegetation types. In contrast, *B. maculata* and *P. lucasii* were not observed in the orchards. In the oil palm plantations, the capture rate of *C. brachyotis* I was notably higher than in the other vegetation types. However, the capture rates of other bat species were low or zero in the plantations.

Cynopterus brachyotis I was the most common and abundant species in all vegetation types, except for primary forests. This species accounted for 44% of all bats in the secondary forests, 41% in the orchards, and 72% in the oil palm plantations. In the primary forests, the dominant species was *H. cervinus* (24% of all bats), and *C. brachyotis* I accounted for only 4% of bats.

Simpson's index of diversity also varied among plant communities (Table 2). Simpson's index for megachiropterans was highest in primary forests, lower in secondary forests and orchards, and lowest in the oil palm plantations.

Discussion

To our knowledge, ours is the first study to demonstrate clear differences in the diversity and abundance of bats among forests and agricultural lands within a single landscape in Southeast Asia. The study showed that the diversity and abundance of bats are strikingly different among different vegetations in spite of a relatively small study area. In addition, the observed movements and feeding habits demonstrated potential effects of agricultural lands on nearby forests or other vegetation.

Relative to primary and secondary forests, the number of megachiropteran species was somewhat low in orchards and notably low in the oil palm plantations. In addition, the capture rates of two frugivorous bats (*B. maculata* and *P. lucasii*) clearly differed among the four vegetation types, and the two species were not recorded in the orchards or the oil palm plantations. These results indicate that megachiropteran species rarely use agricultural lands for feeding; thus, the vegetation is not suitable for maintaining a diversity of megachiropterans. Moreover, there may be no species unique to agricultural lands because those species for which more than two individuals were captured were also recorded at least once in the primary or secondary forests. Megachiropteran diversity in natural forests is thought to be maintained by various factors, including the diversity of food sources (Hall et al. 2004; Hodgkison et al. 2004a), the availability of roosts for tree-roosting bats (Zubaid 1993), and the heterogeneity of forest structure (Hall et al. 2004; Hodgkison et al. 2004b). The oil palm plantations clearly lacked these characteristics.

The capture rate of megachiropterans differed significantly among vegetation types, with high rates in the oil palm plantations and orchards and low rates in the secondary and primary forests. This trend was nearly opposite to the pattern of bat diversity; diversity was quite low in plantations, low in orchards and relatively high in the secondary and primary forests. The observed high capture rate and low species diversity of megachiropterans in agricultural lands were similar to the pattern reported by Hall et al. (2004), although their sampling sites were scattered across Southeast Asia and the crop species studied differed from those in our study. In the Philippines, Heaney et al. (1989) also measured high capture rates and low diversity of megachiropterans in agricultural lands relative to lowland forests. Thus, these patterns may be common throughout Southeast Asia. Similar patterns have also been found in the Neotropics. For example, Medellin et al. (2000) observed both low species richness of phyllostomid bats and high dominance of particular bat species in agricultural lands (shaded cacao plantations and cornfields with other crops) compared to adjacent rainforests. Phyllostomid bat diversity was also low in coffee plantations relative to small fragmented forests (Numa et al. 2005).

Microchiropterans showed a pattern quite different from megachiropterans. The capture rate of microchiropterans was lower in the oil palm plantations but did not differ among the other three vegetation types. One possible cause of the strikingly low capture rate of microchiropterans in the oil palm plantations may have been the low abundance of insects. Although studies concerning the abundance of prey insects in this vegetation type are rare, Chung et al. (2000) reported low beetle abundance and diversity in oil palm plantations compared to logged and primary forests in Sabah, Malaysia.

The drastic increase of *C. brachyotis* I in the oil palm plantations was a particularly intriguing result. The population size of *C. brachyotis* I in the oil palm plantations may indeed be large, considering that its capture rate was extraordinarily high and the plantations cover a vast area (ca. 388,500 ha in Sarawak as of 2004; Brown and Jacobson 2005). High capture rates and dominance of *C. brachyotis* in agricultural lands have been reported repeatedly in other regions (Abdullah et al. 1997; Hall et al. 2004). For example, Abdullah et al. (1997) measured a very high capture rate of *C. brachyotis* (570 individuals per 10,000 m^2h) in *Cocos* and *Musa* plantations in Indonesia, where *C. brachyotis* accounted for 93% of total captures. We collected seeds of forest plants from individuals captured in the plantation. The results indicate that the species move into, and affect, adjacent forests, although the intensity of the effects is unknown.

References

Abdullah MT. 2003. Biogeography and variation of Cynopterus brachyotis in Southeast Asia. PhD thesis. The University of Queensland, Australia.

Abdullah MT, Moritz C, Grigg GG, Hall LS. 2000. Evidence of cryptic species within Cynopterus brachyotis using mtDNA sequence. In: Yaacob Z, Moo-Tan S, Yorath S (eds) Proceedings of the international conference on in-situ and ex-situ biodiversity conservation in the new millennium. Yayasan Sabah, Kota Kinabalu, pp. 403–408

Abdullah MT, Siswanto H, Widiyanto A, Setiabudi A, Firmansyah. 1997. Abundance, diversity and distributional records of bats in disturbed habitats in Kalimantan Barat, Indonesia. Sarawak Mus J 11:75–84

Altringham JD. 1996. Bats: biology and behaviour. Oxford University Press.

Brown E, Jacobson MF. 2005. Cruel oil: how palm oil harms health, rainforest and wildlife. Center for Science in the Public Interest. Washington, DC.

Chung AYC, Eggleton P, Speight MR, Hammond PM, Chey VK. 2000. The diversity of beetle assemblages in different habitat types in Sabah, Malaysia. Bull Entomol Res 90:475–496

Corbet GB, JE Hill. 1992. Order Chiroptera. In: Corbet GB, Hill JE (eds) The mammals of the Indomalayan region. Oxford University Press, Oxford, pp. 54–161

Francis CM. 1990. Trophic structure of bat communities in the understory of lowland dipterocarp rain forest in Malaysia. J Trop Ecol 6:421–431

Fujita MS, Tuttle MD. 1991. Flying foxes (Chiroptera: Pteropodidae): threatened animals of key ecological and economic importance. Conserv Biol 5:454–463

Hall LS, Grigg GG, Moritz C, Ketol B, Sait I, Marni W, Abdullah MT. 2004. Biogeography of fruit bats in Southeast Asia. Sarawak Mus J 80:191–284

Heaney LR, Heideman PD, E. Rickart A, Utzurrum RB, Klompen JSH. 1989. Elevation zonation of mammals in the central Philippines. J Trop Ecol 5:259–280

Hodgkison R, Balding ST, Zubaid A, Kunz TH. 2004a. Temporal variation in the relative abundance of fruit bats (Megachiroptera: Pteropodidae) in relation to the availability of food in a lowland Malaysian rain forest. Biotropica 36:522–533

Hodgkison R, Balding ST, Zubaid A, Kunz TH. 2004b. Habitat structure, wing morphology, and the vertical stratification of Malaysian fruit bats (Megachiroptera: Pteropodidae). J Trop Ecol 20:667–673

Jones G, Rydell J. 2002. Attack and defense: interactions between echolocating bats and their insect prey. In: Kunz TH, Fenton MB (eds) Bat ecology. The University of Chicago Press, Chicago, pp. 301–345

Kunz TH. 1988. Age determination in bats. In: Kunz TH (ed) Ecological and behavioral methods for the study of bats. Smithsonian Institution Press, Washington, D.C., pp. 47–58

Marshall AG. 1985. Old World phytophagous bats (Megachiroptera) and their food plants: a survey. Zool J Linn Soc 83:351–369

Medellin RA, Equihua M, Amin MA. 2000. Bat diversity and abundance as indicators of disturbance in neotropical rainforest. Conserv Biol 14:1666–1675

Nakagawa M, Miguchi H, Nakashizuka T. 2006. The effects of various forest uses on small mammal communities in Sarawak, Malaysia. For Ecol Management 231:55–62

Numa C, Verdú JR, Sánchez-Palomino P. 2005. Phyllostomid bat diversity in a variegated coffee landscape. Biol Conserv 122:151–158

Payne J, Francis CM. 1998. A field guide to the mammals of Borneo. The Sabah Society, Kota Kinabalu, Sabah, Malaysia.

Primack R, Corlett R. 2005. Tropical rain forests: an ecological and biogeographical comparison. Blackwell Publishing, Oxford.

Racey PA, Entwistle AC. 2002. Conservation ecology of bats. In: Kunz TH, Fenton MB (eds) Bat ecology. The University of Chicago Press, Chicago, pp 680–743

Shanahan M, Debski I. 2002. Vertebrates of Lambir Hills National Park, Sarawak, Malaysia. Malaysian Nat J 56:103–118

Simpson EH. 1949. Measurement of diversity. Nature 163:688

Sokal RR, Rohlf FJ. 1973. Introduction to Biostatistics. W. H. Freeman and Company, San Francisco and London. Wright SJ. 2005. Tropical forests in a changing environment. Trends Ecol Evol 20:553–560

Zhao S, Peng C, Jiang H, Tian D, Lei X, Zhou X. 2006. Land use change in Asia and the ecological consequences. Ecol Res 21:890–896

Zubaid A. 1993. A comparison of the bat fauna between a primary and fragmented secondary forest in Peninsular Malaysia. Mammalia 57:201–206

Table 1 Capture rate of bats (individuals per 10,000m2h). Data for species with small sample sizes (<20 individuals) are not shown. The rightmost column presents results of the Chi-squared test: *P < 0.05, **P < 0.01, ***P < 0.001, –: impossible to test due to small sample size.

	Primary forests	Secondary forests	Orchards	Oil palm plantations	Significance
Megachiropterans	55	86	208	272	***
Microchiropterans	40	26	49	6	**
Each species					
Megachiroptera					
Balionycteris maculata	16	2	0	0	-
Cynopterus brachyotis I	4	49	106	201	***
C. brachyotis II	8	11	6	9	-
Eonycteris spelaea	4	1	44	12	***
Macroglossus minimus	5	3	31	0	***
Penthetor lucasii	13	3	0	0	-
Microchiroptera					
Hipposideros cervinus	23	13	11	3	*
Glischropus tylopus	4	1	31	0	-

Table 2 Simpson's index of diversity in the four vegetation types. (N: number of individuals, S: number of species, 1/D: Simpson's index of diversity). 1/D was not calculated for microchiropterans in oil palm plantations due to the small sample size.

		Primary forests	Secondary forests	Orchards	Oil palm plantations
Megachiropterans	*N*	78	75	136	92
	S	8	10	6	3
	1/*D*	5.00	2.09	2.54	1.19
Microchiropterans	*N*	57	23	32	2
	S	11	8	5	2
	1/*D*	2.95	3.50	2.24	
All bats	*N*	135	98	168	94
	S	19	18	11	5
	1/D	7.86	3.38	3.65	1.24

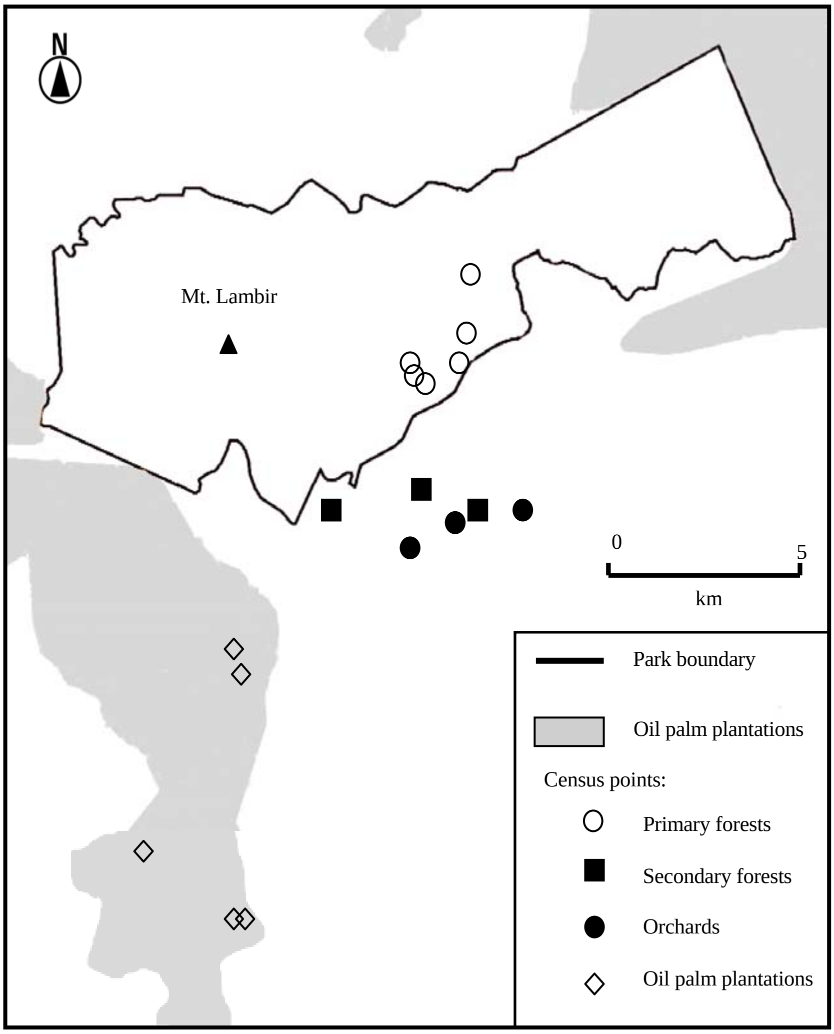


Figure 1. Study area and location of census points.

Effect of Forest Use on Community Structures and Trophic Levels of Small Mammals in Sarawak, Malaysia

Michiko Nakagawa[1, 3], Hideo Miguchi[2], Fujio Hyodo[1], Tohru Nakashizuka[1, 4]
[1] *Research Institute for Humanity and Nature*
[2] *Faculty of Agriculture, Niigata University*
[3] *Current address; Graduate School of Bioagricultural Sciences, Nagoya University*
[4] *Current address; Graduate School of Life Sciences, Tohoku University*

Introduction

In terrestrial forest ecosystems, anthropogenic effects on biodiversity and ecosystem function are a global concern. Tropical rain forests in Southeast Asia are characterized by extremely high plant diversity (Whitmore 1984); however, these forests have been exposed to drastic and rapid deforestation because of an increasing human population and a developing economy. The effect of altering habitats in tropical forests on the biodiversity of wild animals is an urgent subject for species conservation and proper forest management. Much research has been conducted on the effects of deforestation or forest use on changes in wildlife species composition or abundance, such as that for logged forests (Willott et al. 2000), fragmented forests (Harrington et al. 2001), and fallows (Medellín and Equihua 1998). In reality, however, a landscape is a complex assemblage of these forest types. To clearly understand the effects of forest use on the community structure in a region, we must asses a complex assemblage of these diverse vegetation types. The alternation of plant species composition and tree size in degraded forests may also be accompanied by changes in food resource availability for wild animals, resulting in changes in their food intake (Panasci and Whitacre 2000). The analysis of stable nitrogen isotopes (δ15N) in organisms is a powerful tool with which to investigate trophic positions within a food web.

Our objective was to analyze the distribution patterns and levels of δ15N in small mammals in fragmented primeval forests, abandoned fallows at three different developmental stages, and rubber plantations, as compared with the distribution patterns and δ15N levels in primary forests to determine the effects of human forest use on small mammal communities and trophic levels in Sarawak, Malaysia. Because small mammals play an important role as seed dispersers in forest ecosystems and form the base of the mammalian food chain (Shine et al. 1998; Shanahan and Compton 2000), understanding the effects of forest uses on small mammals is needed to determine proper forest management strategies.

Materials and Methods

Study site

A field survey was conducted during August–September 2003, June – July 2004, and January–March 2005 in and around Lambir Hills National Park (LHNP), Sarawak, Malaysia (4°2’N, 113°5’E). The study site contained 20 plots (each 10×100 to 10×200 m) in six forest types: primary forests (two plots), fragmented primeval forests (six plots), abandoned fallows at three different developmental stages (new, young, and old: three plots each), and rubber plantation (three plots). Light conditions on the forest floor were determined by quantifying the percent canopy openness using a digital camera with a fisheye lens (Cool Pix 910, Nikon).

Small mammal trapping and litter sampling

Small mammals were live-trapped for five consecutive days and nights using wire-mesh live cage traps (15×12×30 cm). One trapping series was conducted in each study plot during each field survey for a total of three trapping series. Traps were baited with banana and sweet potato and covered with a sheet of transparent plastic to exclude rainfall. We checked all traps once a day in the morning (0700–1100 hours). Captured individuals were identified according to Payne et al. (1985) and marked by toe clipping with a unique number at the site of capture. After marking and recording sex, weight, reproductive condition, and capture point, individuals were released. Nomenclature follows that of Payne et al. (1985). The clipped toes were immediately returned to the laboratory, stored in a freezer at –20°C, oven-dried at 50°C for 48 h, and kept dry until analysis.

Leaf litter samples were hand-collected from ten 15×15 cm quadrats at 10-m intervals within each plot. These were oven-dried at 50°C for 72 h and ground to fine powder after removing sand and fungi from the leaves, twigs, and petioles.

Isotope analysis

For the δ15N analysis, samples from 144 individuals, representing 19 species in 3 families (treeshrews, squirrels, and rats or mice) were folded into tin capsules. The level of δ15N was measured using a mass spectrometer (Delta S or Delta plus XP, Germany) coupled with an Elemental Analyzer (Flash EA 111). The natural abundance of ^{15}N is expressed in per mil (‰) deviation from an international standard: $\delta^{15}N = (R_{sample}/R_{standard} - 1) \times 1000$, where R is $^{15}N/^{14}N$. Atmospheric N was used as the N standard.

Data analysis

We conducted detrended correspondence analysis (DCA) on the species abundance data ($N \geq 5$) to identify gradients in the small-mammal assemblages over the 20 study plots. Less common species (N of captures <5) were excluded from the DCA. To correct for differences in the δ15N of primary production among sites, we calculated the average $\delta^{15}N$ of 10 leaf litter samples from each plot and estimated the corrected $\delta^{15}N$ of small mammals as: $\delta^{15}N_{\text{corrected}} = \delta^{15}N_{\text{small mammal}} - \delta^{15}N_{\text{leaf litter}}$, where $\delta^{15}N_{\text{small mammal}}$ is the directly measured $\delta^{15}N$ of a small mammal at a particular site and $\delta^{15}N_{\text{leaf litter}}$ is the average $\delta^{15}N$ of 10 leaf litter samples from that plot. DCA was performed using CANOCO 4 (ter Braak and Smilauer 1998) and all other statistical analyses were performed using JMP 4.0 (SAS Institute 2000).

Results

Community structures of small mammals

In total, we captured 283 individuals of 22 species from four families: Tupaiidae, Muridae, Sciuridae, and Erinaceidae. DCA extracted two axes that cumulatively accounted for 45.7% of the variation in the small mammal species abundance data. The first axis (DC1) was significantly correlated with the abundance of *Niviventer cremoriventer*, *Sundamys muelleri*, and *Leopoldamys sabanus*, whereas the second axis (DC2) was significantly correlated with the abundance of *Tupaia minor*, *T. gracilis*, and *Rattus rattus*; both correlations were positive. DC2 was also significantly negatively correlated with the abundance of *Maxomys rajah*. The DCA showed two distinctive groups: new fallow and other forest types (Fig. 1). The three new fallow plots had a high abundance of two species of treeshrew and house rat (*R. rattus)*. However, there was no obvious partitioning among the other five forest types.

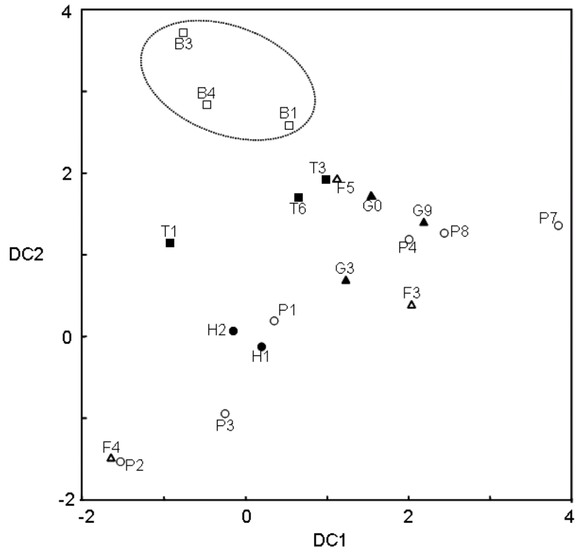


Fig. 1 Plot of the first two axes of the detrended correspondence analysis of the small mammal community species-abundance data ($N \geq 5$) from 20 study plots. Symbols indicate the forest type: solid circle, primary forest; open circle, fragmented primeval forest; solid square, young fallow; open square, new fallow; solid triangle, rubber plantation; and open triangle, old fallow; after Nakagawa et al. (2006).

Trophic levels of small mammals

Differences in the $\delta^{15}\mathrm{N}$ of leaf litter among the forest types were small and not significant. The $\delta^{15}\mathrm{N}_{\mathrm{corrected}}$ of rats and mice differed significantly among forest types (ANOVA, $F = 10.83$, $P < 0.0001$); those in forests that had been used relatively recently, such as new and young fallow and rubber plantations, had significantly higher levels of $\delta^{15}\mathrm{N}_{\mathrm{corrected}}$ than did those in primary or fragmented forests and old fallow (Fig. 2). In contrast, we found no significant differences in levels of $\delta^{15}\mathrm{N}_{\mathrm{corrected}}$ for treeshrews and squirrels among forest types. Canopy openness differed significantly among forest types (ANOVA, $F = 8.32$, $P < 0.001$) and was highest in new fallow. For the squirrel *Callosciurus notatus* and two rat species, *M. rajah* and *N. cremoriventer*, canopy openness and $\delta^{15}\mathrm{N}_{\mathrm{corrected}}$ were significantly and positively correlated (Fig. 3); such a pattern was not found for lesser (*T. minor*) or large (*T. tana*) treeshrews.

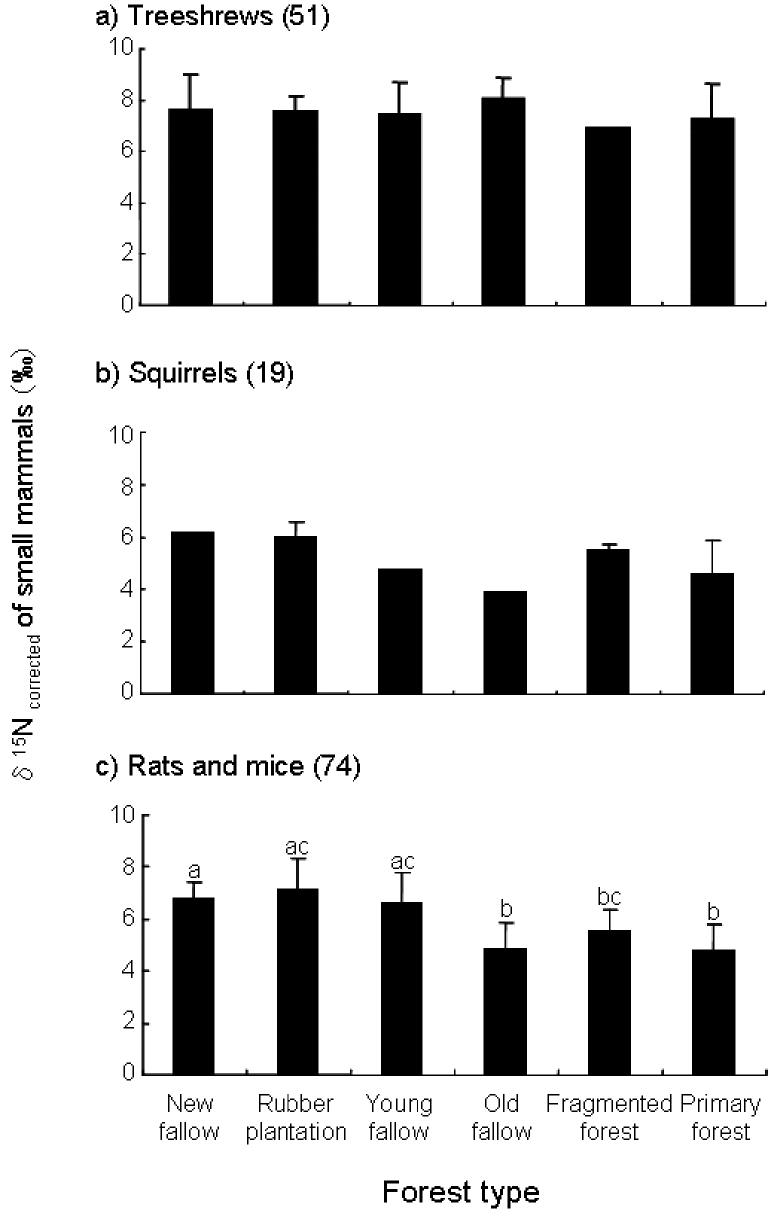


Fig. 2 δ^{15}N $_{\text{corrected}}$ of (a) treeshrews, (b) squirrels, and (c) rats and mice among six forest types. Bars sharing any letters are not significantly different among the forest types (Scheffe's multiple comparison, $P < 0.05$). No significant differences were detected in treeshrews and squirrels. Sample sizes are indicated in parentheses; after Nakagawa et al. (2007).

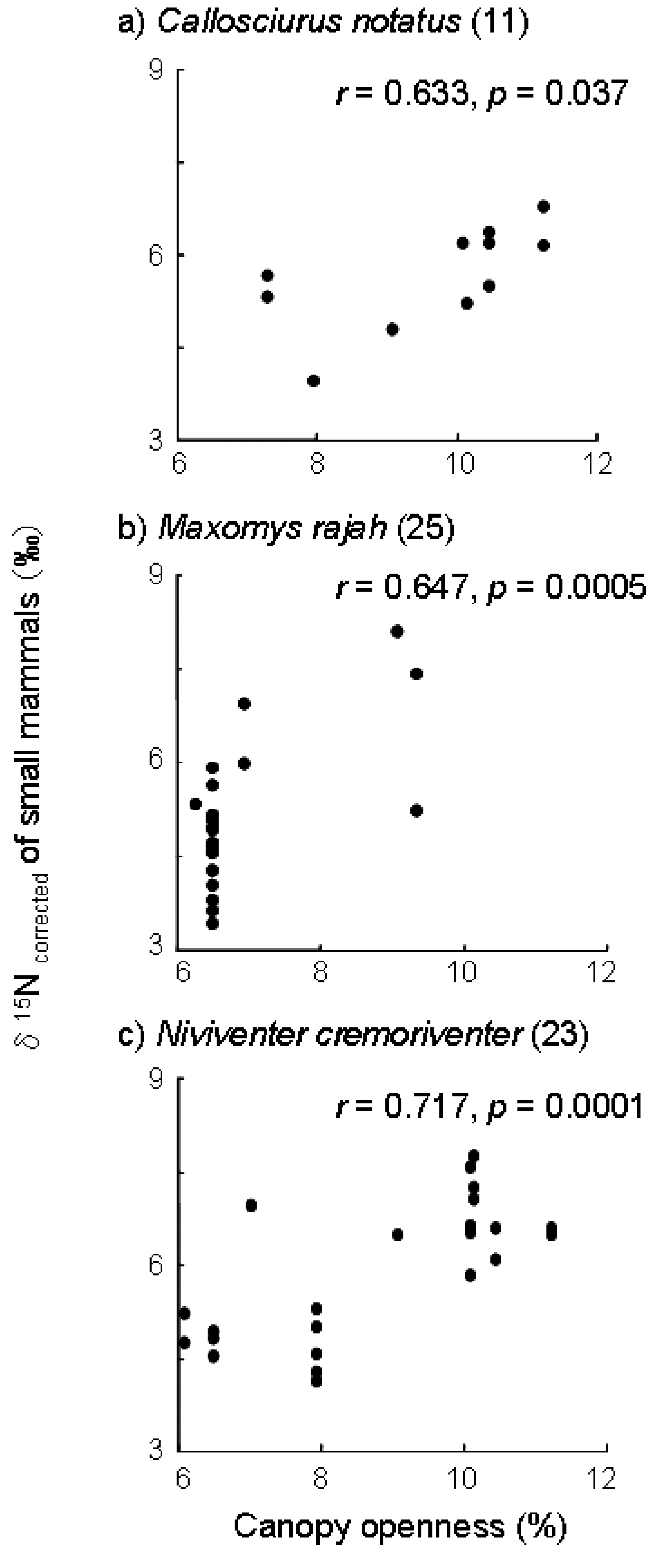


Fig.3 Correlations between canopy openness and the $\delta^{15}N_{\text{corrected}}$ of (a) *Callosciurus notatus*, (b) *Maxomys rajah*, and (c) *Niviventer cremoriventer*. Sample sizes are shown in parentheses; after Nakagawa et al. (2007).

Discussion

The analysis of small mammal species abundance among forest types indicated two distinct groups. Small mammal communities in new fallow had comparatively more house rats and lesser and slender treeshrews. The house rat is a human-associated species that is adapted to disturbance (Lynam and Billick 1999). The high occurrence of rats in new fallow, where disturbance had recently occurred and recovery from the disturbance had not yet advanced, is not unexpected. Lesser and slender treeshrews occur in gardens and plantations, as well as in forests (Payne et al. 1985), suggesting that they could be adaptable to disturbed habitats. Our results also indicate that small mammal communities could recover from the disturbance relatively quickly, that is, within several years after slash-and-burn agriculture. Although the species composition of young fallow was not entirely similar to that of primary forest, the plots in young fallow were included in the same group as primary forest; moreover, these plots contained some interior forest specialists or dominant species in the primary forest, such as Muller’s rat and brown spiny rat, which showed significant correlations with the DCA axes.

Rats and mice in highly degraded forests (e.g., new and young fallow and rubber plantation) were more enriched in 15N compared to those in primary forests or somewhat degraded forests (e.g., old fallow and fragmented primeval forest). This result indicates that rats and mice in highly degraded forest are likely to feed on more consumers such as invertebrates rather than on plant organs compared with rodents in primary or somewhat degraded forests. On the other hand, the δ15N of treeshrews did not differ among forest types, suggesting that their diet was not affected by forest uses. This result indicates that treeahrews that normally prefer to consume insects may be less affected by insect abundance as a result of changes in forest uses. The trend was also supported at the species level, except for treeshrews. Two species of rat and one species of squirrel showed significantly higher δ15N in forests with high compared to low canopy openness, suggesting an increase in consumers in their diets. In disturbed forests, which have high canopy openness, insects such as flies and butterflies were abundant (Willott et al. 2000; Horn et al. 2005). Rats and mice in the region are thought to consume a wide range of fruits or seeds, as well as insects (Payne et al. 1985; Kitamura et al. 2006). When insects are abundant, they may be easy to capture as a food resource. Direct quantification of diet or gut contents analysis of small mammals in a future study would clearly support our hypothesis.

Given the variation in the food supply and changes in vegetation cover that accompany habitat disturbances, dietary flexibility would be crucial for survival in degraded forests. The dietary flexibility of rats and squirrels may be the reason why community structures of small mammals are similar among various forest types examined in this study (except for new fallow). However, changes in the feeding activity of small mammals associated with forest uses may affect the distribution or survival of insects through higher predation pressure, as well as the regenerating patterns of plants through decreased predation and dispersal of fruits.

Acknowledgments

We thank L. Chong (Sarawak Forestry Corporation) and J. Kendawang (Forest Department Sarawak) for allowing us access to the study site and supporting our research in Sarawak. The hospitable support and cooperation of the inhabitants at Rh. Aji, Rh. Bundan, and Nakat was most helpful. The field survey was kindly supported by K. Hamamoto, K. Takahashi, S. Kitamura, T. Matsumoto, Jinggan, Ekeh, Umping, and

Maok. Financial support was provided by RIHN research projects 2-2 and 3-1 and JSPS Research Fellowships for Young Scientists to M. N. and F. H.

References

Harrington GN, Freeman AND, Crome FHJ (2001) The effects of fragmentation of an Australian tropical rain forest on populations and assemblages of small mammals. J Trop Ecol 17:225-240

Horn S, Hanula JL, Ulyshen MD, Kilgo JC (2005) Abundance of green tree frogs and insects in artificial canopy gaps in a bottomland hardwood forest. Am Midl Nat 153:321-326

Kitamura S, Suzuki S, Yumoto T, Poonswad P, Chuailua P, Plongmai K, Maruhashi T, Noma N, Suckasam C (2006) Dispersal of Canarium euphyllum (Burseraceae), a large-seeded tree species, in a moist evergreen forest in Thailand. J Trop Ecol 22:137-146

Lynam AJ, Billick I (1999) Differential responses of small mammals to fragmentation in a Thailand tropical forest. Biol Conserv 91:191-200

Medellín RA, Equihua M (1998) Mammal species richness and habitat use in rainforest and abandoned agriculture fields in Chiapas, Mexico. J Appl Ecol 35:13-23

Nakagawa M, Miguchi H, Nakashizuka T (2006) The effects of various forest uses on small mammal communities in Sarawak, Malaysia. For Ecol Manage 231:55-62

Nakagawa M, Hyodo F, Nakashizuka T (2007) Effect of forest use on trophic levels of small mammals: an analysis using stable isotopes. Can J Zool 85:472-478

Panasci T, Whitacre D (2000) Diet and foraging behavior of nesting roadside hawks in Petén, Guatemala. Wilson Bull. 112:555-558

Payne J, Francis CM, Phillipps K (1985) A Field Guide to the Mammals of Borneo. The Sabah Society, Kota Kinabalu, Malaysia

SAS Institute (2000) JMP user’s guide. SAS Institute, Cary, NC, USA

Shanahan M, Compton SG. (2000) Fig-eating by Bornean tree shrews (Tupaia spp.): evidence for a role as seed dispersers. Biotropica 32:759-764

Shine R, Harlow PS, Keogh JS, Boeadi (1998) The influence of sex and body size on food habits of a giant tropical snake, Python reticulates. Func Ecol 12:248-258

ter Braak CJF, Smilauer P (1998) CANOCO reference manual and user’s guide to Canoco for windows: software for canonical community ordination (version 4). Microcomputer Power, Ithaca, New York, USA

Whitmore TC (1984) Tropical rain forests of the Far East. 2nd ed. Clarendon, Oxford

Willott SJ, Lim DC, Compton SG., Sutton SL (2000) Effect of selective logging on the butterflies of a Bornean rainforest. Conserv Biol 14:1055-1065

Community Structure of Aphyllophoraceous Fungi and Impacts of Human Forest Use in Sarawak, Malaysia

Satoshi Yamashita[1], Tsutomu Hattori[2], Kuniyasu Momose[3], Michiko Nakagawa[4], Masahiro Aiba[5], Tohru Nakashizuka[5]
[1]*Research Institute for Humanity and Nature*
[2]*Forestry and Forest Products Research Institute*
[3]*Ehime University*
[4]*Nagoya University*
[5]*Tohoku University*

Introduction

Fungi play important roles as decomposers and symbionts of plants in forest ecosystems and should contain enormously diverse species. Hawksworth and Mueller (2005) estimated the number of fungal species to be between 600 000 and 1 500 000 and suggested that there are hot spots of fungal diversity in the tropical region. Pegler (1997) also pointed out that Southeast Asia has possibly the greatest species diversity of fungi. However, information on the fungal community structure in the tropical forest is almost limited to that of Aphyllophorales in the neotropical region (Ferrer and Gilbert 2003; Schmit 2005; but see Hattori and See 2003). In addition, natural forests, which would support high fungal species diversity, are disappearing at an annual rate of 1.4% in Southeast Asia (Sodih et al. 2004). Thus, reliable and rapid evaluation of fungal community structures under undisturbed conditions in Southeast Asia is greatly needed.

It is well known that fungal flora and their species diversity are affected by forest environmental factors (e.g., Yamashita and Hijii 2006). Human uses of the forest affect the composition and diversity of macrofungi, such as Agaricales and Aphyllophorales in temperate and boreal zones (e.g., Penttilä et al. 2004; Sippola et al. 2004). Forest use in tropical regions is also expected to affect fungal community structure, although studies on the effect of forest use on the fungal community structure in this region are rare.

Although long-term study is needed to adequately characterize the fungal flora (Mueller et al. 2004), rapid assessments are still helpful for monitoring and managing rapidly changing tropical forests. Because fruiting bodies of bracket fungi and shelf fungi (Aphyllophorales, Basidiomycetes), which mainly utilize fallen logs, twigs, and other woody materials as their substrates, are long-lasting, year-to-year variation in species composition is smaller than that of ephemeral fungi, such as agaric mushrooms (Berglund et al. 2005). Aphyllophorales has also been taxonomically well described; thus, it provides suitable candidates of environmental indicators that could be used to examine the effects of forest use in tropical regions.

In this study, we conducted field survey and estimated the number of fungal species to reveal the community structure of aphyllophoraceous fungi in the primary forest. We compared fungal species density and species composition among five forest types (rubber plantation, land left fallow after rice cultivation for less than 10 years, land left fallow after rice cultivation for more than 30 years, isolated and fragmented primary forest, and primary forest) to reveal the effects of human forest use on the aphyllophoraceous fungal community

Methods

The study area is located in and around Lambir Hills National Park, Sarawak, Malaysia (4º20'N, 113º50'E). Aphyllophorales is a taxonomically polyphyletic group, but almost all species are pathogenic or saprotrophic fungi. We focused on polypores, hydnoid fungi, and stereoid fungi, but we excluded clavarioid and corticioid fungi because it is very difficult to identify species in these two groups. After identifying the fungi to the species or morphospecies level, we collected fruiting bodies and preserved them as dried specimens at the Sarawak Forestry Corporation (Forest Research Center) in Kuching, Sarawak, Malaysia.

To reveal the aphyllophoraceous fungal community structure in the primary forest, we established twelve 100×10 m transects which is divided into forty 5×5 m quadrats in the park. We collected all fruiting bodies of Aphyllophorales in these transects in June and December 2006. At the same time, we recorded the diameter and decay stage of the coarse woody debris (CWD) on which the fruiting bodies appeared. Because the number of fruiting bodies does not always correspond to the number of fungal individuals, we used the number of CWD on which almost all species occurred as that of individuals. For fruiting bodies of *Amauroderma subrugosum* and other fungi which appeared directly from the ground, we treated the number of 5×5 m quadrats as the number of individuals. We estimated the number of aphyllophoraceous fungal species using three estimators: Chao 1, Chao 2, and Jackknife 1.

To reveal the effects of forest use on the fungal community, we established 10 plots in and around the park. Two plots were established for every forest type: rubber plantation, land left fallow after rice cultivation for less than 10 years, land left fallow after rice cultivation for more than 30 years, isolated and fragmented primary forest, and primary forest. In a primary stand in the park, two study plots were established. The other plots were established around the park. In each plot, we established one long band transect (10 m wide and 100 m long) and recorded tree composition, soil water potential, the degree of canopy openness, and the mass of forest-floor leaf and branch litter (Yamashita et al. in press). Perpendicular to each long transect, we established four shorter band transects (5 m wide and 40 m long) at intervals of 25 or 50 m, in which we recorded the number of CWD pieces. We collected all fruiting bodies that appeared in the short band transects from 17 June to 6 September 2005. We used the number of branch transects in which these species occurred because the number of fruiting bodies does not always correspond to the number of fungal individuals.

We used canonical correspondence analysis (CCA) to reveal the relationships between fungal community composition and the environmental variables (Jongman et al. 1995). We performed preliminary principal-components analysis (PCA) for plant species composition so that we could use the PC axes as factors related to plant species composition. The PCA revealed that plant species composition in the rubber plantation and the young fallow forest differed conspicuously from each other and from that in the other forest types. Differences in the composition of CWD among plots were also evaluated by using the PCA scores, and the PCA results for CWD composition showed no clear grouping. We used the degree of canopy openness, soil water potential, the scores for PC-1 and PC-2 for the composition of CWD, the total number of pieces of CWD, the dry weight of litter, the scores of PC-1 and PC-2 for plant species composition, and the total basal area of the plot as environmental variables in the CCA.

Results

Fungal community structure in the primary forest

A total of 721 individuals from 101 species was collected from the 1.2 ha of transects. The estimated numbers of species were 155 (Chao 1), 161 (Chao 2), and 147 (Jackknife 1). Almost half of these species were singletons. Dominant species were *A. subrugosum*, *Ganoderma austrael*, *Microporus xanthopus*, and *Microporus affinis* (> 40 individuals in total). *Ganoderma australe* tended to appear on large fallen trunks, whereas *M. xanthopus* and *M. affinis* appeared on fallen branches.

Effects of forest use on the fungal community

The environmental variables are shown in Table 1. Canopy openness ranged from 6.1 to 10.5%, soil water potential from -586 to -23 kpa, number of pieces of CWD from 13 to 533 per ha, leaf and branch litter from 0.54 to 1.16 kg dry wt./m^2, and basal area from 11.5 to 74.6 m^2/ha.

A total of 155 samples from 67 species of polypores, hydnoid fungi, and stereoid fungi was collected during the study period. *Amauroderma subrugosum* and *Trametes* cf. *mimites* were dominant species in the study stands. Twenty-eight aphyllophoraceous species appeared in more than two transects, whereas 39 species were singletons.

The species density (number of species per 200 m^2) differed significantly among forest types ($P < 0.05$) but not among site positions ($P > 0.05$), and the interaction between forest type and site position was not significant ($P > 0.05$; Fig. 1). More than four species per 200 m^2 appeared in the rubber plantation, isolated primary forest, and primary forest. The fungal species density increased significantly with increasing number of pieces of CWD in the short band transect (Pearson’s correlation coefficient; $r = 0.842$, $P < 0.05$), but it decreased significantly with increasing PC-2 scores for plant species composition ($r = -0.803$, $P < 0.05$).

In the CCA ordination, the first axis explained 23.1% of the total variance versus 17.4% for the second axis (Fig. 2). Automatic forward selection revealed that only the number of pieces of CWD significantly affected the fungal species composition (Monte Carlo permutation test, $P < 0.05$). The occurrences of *Flabellophora licmophora* (*Flic*), *Coriolopsis retropicta* (*Cret*), *Microporus vernicipes* (*Mver*), and *A. subrugosum* (*Asub*) were positively correlated with the number of pieces of CWD.

Fungal flora in the young fallow forest near the park and the old fallow forest near the village were clearly different from those in the other plots. The other eight plots were clustered at the central part of the diagram. Young fallow forest near the park and old fallow forest near the village were associated with *Podoscypha nitidula* (*Pnit*) and *Podoscypha* sp. 1 (*Psp1*), respectively.

Discussion

Four of the world’s 25 biodiversity hot spots for plants and vertebrates are in Southeast Asia (Myers et al. 2000), and the study area is famous for its diverse plant species. Our study revealed that primary forest in this region maintains a high species diversity of Aphyllophorales compared with temperate and boreal zones

(10 to 89 species; Schmit 1999; Penttilä et al. 2004).

Community structures of living organisms other than fungi are affected by anthropogenic forest use in tropical regions (Waltert et al. 2003; Yasuda et al. 2003). Schulze et al. (2004) revealed that almost all the species groups or guilds of plants, birds, and insects could not accurately predict the biodiversity of other groups. A previous study at our study site revealed that the diversity of small mammals did not differ clearly among forest types (Nakagawa et al. 2006). Conversely, except at the rubber plantation (which had a high amount of CWD), our study showed that human forest use negatively affected aphyllophoraceous fungal diversity. This suggests that fungal diversity could indicate some aspects of a forest's environmental condition, such as the quantity of CWD. In boreal and temperate regions, the community structure of both ectomycorrhizal fungi (Visser 1995) and wood-inhabiting fungi (Penttilä et al. 2004) were strongly affected by forest management. Iwabuchi et al. (1994) showed that the species diversity of macrofungi increased as successional changes occurred in plant species composition. Hence, the response of the species diversity of macrofungi to forest disturbance appears to not differ greatly among regions.

Recently, studies of the community structure of decomposers have stressed the importance of management of CWD to maintain species richness of saproxylic fungi and other living organisms (Jonsson and Kruys 2001; Lindhe et al. 2004; Jonsson et al. 2005) because the abundance of CWD is positively correlated with the species richness of both rare and common wood-inhabiting fungi (Penttilä et al. 2004). Our results also showed that the abundance of CWD affected the species richness and composition of aphyllophoraceous fungi in the stands. This suggests that overuse of CWD in tropical regions, such as harvesting it for fuel, would negatively affect the fungal community that develops on CWD, with consequences such as a loss of species diversity and of functional groups.

Species diversity of the Aphyllophorales reflected forest conditions, especially the quantity of CWD, but species diversity might not be the best indicator of forest conditions in very different habitats. In our study, tree composition differed between rubber plantations and the other types of forest, but the number of fungal species was similar to those in isolated primary forests and primary forests. Some species, such as *A. subrugosum*, which appeared mainly in primary and isolated primary forests and was one of the dominant species in the primary forest, could be good indicators of forest condition. Both species diversity and the presence of indicator species have shortcomings, and further study is needed to elucidate good indicators of forest condition.

Acknowledgments

We thank L. Chong (Sarawak Forestry Corporation, SFC) and J. Kendawang (Forest Department Sarawak) for their permission to conduct research in the study area. S. M. Hang (SFC) helped us sort the fungal fruiting bodies. We are also grateful to the inhabitants at Rh. Chabu and Rh. Bundan for their helpful support; in particular, Jugok and Ekeh helped us in the field. K. Yokoyama (Shiga University) and A. Kohzu (Kyoto University) provided valuable suggestions for mycological research in tropical regions. We also thank T. Matsumoto, S. Tsuji, and other researchers in the Lambir Hills National Park for their support. This study was supported by RIHN research project 2-2.

References

Berglund H, Edman M, Ericson L (2005) Temporal variation of wood-fungi diversity in boreal old-growth forests: implications for monitoring. Ecol. Appl. 15:970–982

Ferrer A, Gilbert GS (2003) Effect of tree host species on fungal community composition in a tropical rain forest in Panama. Divers. Distrib. 9:455–468

Hattori T, See LS (2003) Community structure of wood-decaying Basidiomycetes in Pasoh. In: Okuda T, Manokaran N, Matsumoto Y, Niiyama K, Thomas SC (Eds) Pasoh; Ecology of a lowland rain forest in Southeast Asia. Springer, Berlin, pp. 161–170

Hawksworth DL, Mueller GM (2005) Fungal communities: Their diversity and distribution. In: Dighton J, White JF, Oudemans P (Eds) The fungal community — Its organization and role in the ecosystem. Taylor & Francis, Boca Raton, USA, pp. 27–37

Iwabuchi S, Sakai S, Yamaguchi O (1994) Analysis of mushroom diversity in successional young forests and equilibrium evergreen broad-leaved forests. Mycoscience 35:1–14

Jonsson BG, Kruys N (2001) Ecology of woody debris in boreal forests. Ecological Bulletins, Lund, Sweden

Jonsson BG, Kruys N, Ranius T (2005) Ecology of species living on dead wood — lessons for dead wood management. Silva Fenn. 39:289–309

Jongman RHG, ter Braak CJF, van Tongeren OFR (1995) Data analysis in community and landscape ecology. Cambridge University Press, Cambridge, UK

Lindhe A, Åsenblad N, Toresson HG (2004) Cut logs and high stumps of spruce, birch, aspen and oak — nine years of saproxylic fungi succession. Biol. Conserv. 119:443–454

Mueller GM, Bills GF, Foster MS (2004) Biodiversity of Fungi: Inventory and monitoring methods. Elsevier, London, UK

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403:853–858

Nakagawa M, Miguchi H Nakashizuka T (2006) The effects of various forest uses on small mammal communities in Sarawak, Malaysia. For. Ecol. Manage. 231:55–62

Pegler DN (1997) The larger fungi of Borneo. Natural History Publications, Kota Kinabalu, Malaysia

Penttilä R, Siitonen J, Kuusinen M (2004) Polypore diversity in managed and old-growth boreal *Picea abies* forests in southern Finland. Biol. Conserv. 117:271–283

Schmit JP (1999) Macrofungal diversity of a temperate oak forest: a test of species richness estimator. Can. J. Bot. 77:1014–1027

Schmit JP (2005) Species richness of tropical wood-inhabiting macrofungi provides support for species-energy theory. Mycologia 97:751–761

Schulze CH, Waltert M, Kessler PJA, Pitopang R, Shahabuddin, Veddeler D, Mühlenberg M, Gradstein SR, Leuschner C, Steffan-Dewenter I, Tscharntke T (2004) Biodiversity indicator groups of tropical land-use systems: comparing plants, birds, and insects. Ecol. Appl. 14:1321–1333

Sippola AL, Similä M, Mikkönen M, Jokimäki J (2004) Diversity of polyporous fungi (Polyporaceae) in northern boreal forests: effects of forest type and logging intensity. Scand. J. For. Res. 19:152–163

Sodih NS, Koh LP, Brook BW, Ng PKL (2004) Southeast Asian biodiversity: an impending disaster. Tends Ecol. Evol. 19:654–660

Visser S (1995) Ectomycorrhizal fungal succession in jack pine stands following wildfire. New Phytol. 129:389–401

Waltert M, Mardiastuti A, Mühlenberg M (2003) Effects of land use on bird species richness in Sulawesi, Indonesia. Conserv. Biol. 18:1339–1346

Yamashita S, Hijii N (2006) Spatial distribution of the fruiting bodies of Agaricales in a Japanese red pine (*Pinus densiflora*) forest. J. For. Res. 11:181–189

Yamashita S, Hattori T, Momose K, Nakagawa M, Aiba M, Nakashizuka T (2008) Effects of Forest Use on Aphyllophoraceous Fungal Community Structure in Sarawak, Malaysia. Biotropica. In press.

Yasuda M, Ishii N, Okuda T, Hussein NA (2003) Small mammal community: habitat preference and effects after selective logging. In: Okuda T, Manokaran N, Matsumoto Y, Niiyama K, Thomas SC (Eds) Pasoh; Ecology of a lowland rain forest in Southeast Asia. Springer, Berlin, pp. 533-546

Table 1 Environmental variables for each study plot.

Plot	Position	Canopy Openness (%)	Soil Water Potential (Kpa)	CWD (pieces/ha)	Litter (g dry wt./ m^2)	Basal Area (m^2/ha)
R	Near park	10.5	-582	415	0.98	52.4
	Near village	7.0	-73	295	0.54	54.8
YF	Near park	9.1	-53	165	0.90	11.5
	Near village	9.7	-156	13	0.73	24.7
OF	Near park	7.8	-240	283	0.95	54.2
	Near village	7.7	-545	150	1.16	74.6
IP	Near park	6.1	-586	438	1.08	70.9
	Near village	7.0	-323	305	1.11	74.7
P1	-	6.8	-84	403	1.14	73.4
P2	-	6.9	-23	533	1.16	64.2

Sample statistics except for basal area are means, where number of samples of canopy openness and water potential was 10, that of CWD was 4, and that of litter was 5. After Yamashita et al. (in press).

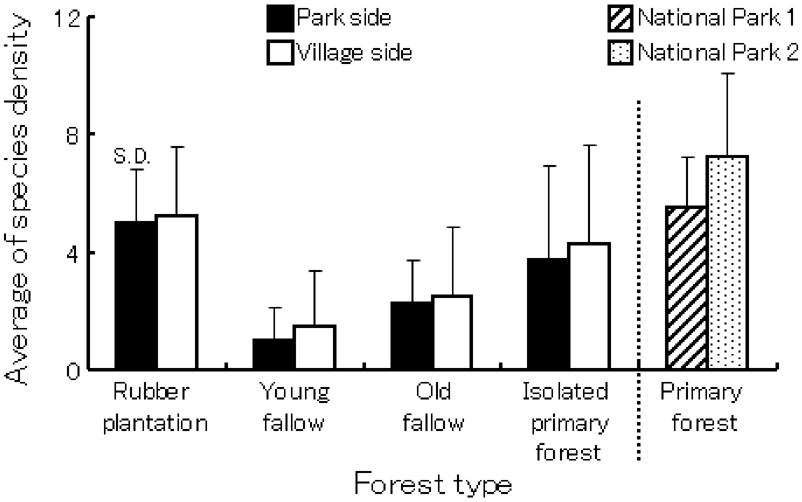


Fig. 1 The average of aphyllophoraceous species density per $200m^2$ found in each plot (n = 4 short band transects). Species density differed significantly differed among forest types (P = 0.007) but not among site positions (park vs. village), and there was no significant site-forest type interaction (two-way ANOVA). The species density in P2 was significantly higher than those in young fallows (one-way ANOVA, Tukey-Kramer's HSD test, $P < 0.05$). After Yamashita et al. (in press).

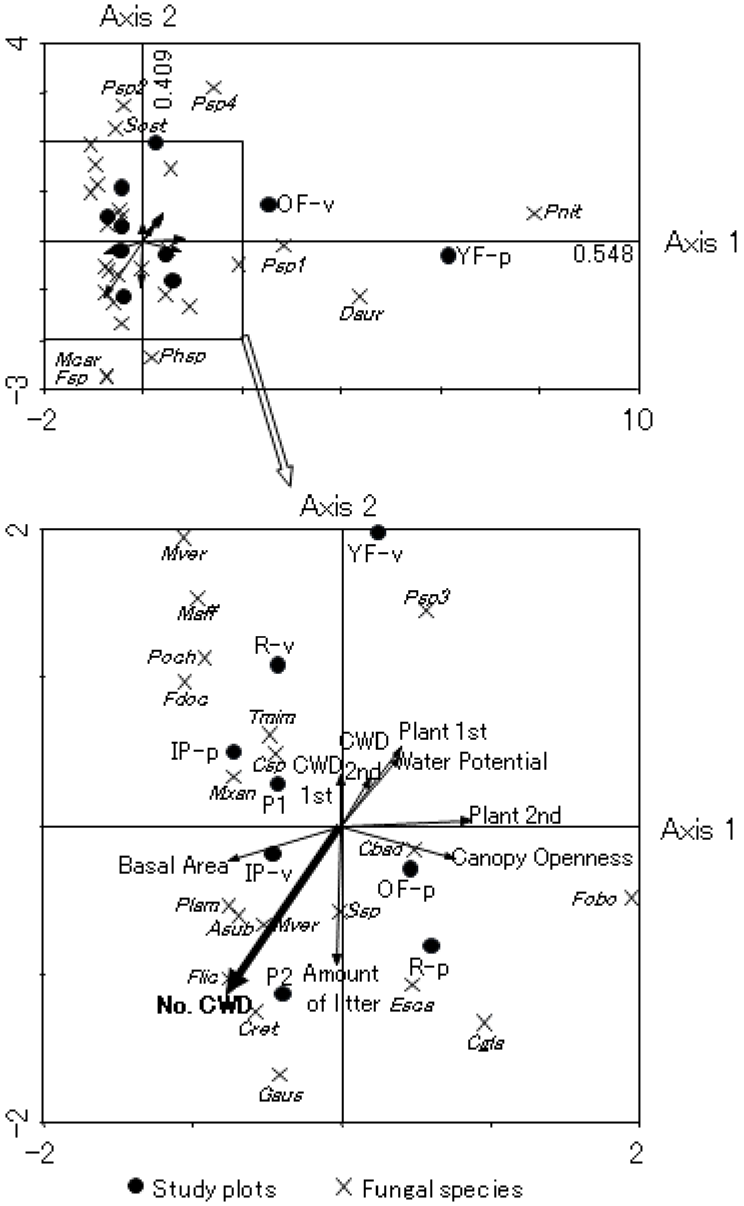


Fig. 2 The results of a canonical correspondence analysis ordination for the site scores of the study plots and the correspondence positions of the aphyllophoraceous species. The upper graph shows all plots and species; the lower graph shows plots and species that lie within 2units on either side of the origin. Environmental variables are shown by arrows. Numbers on the axes are eigenvalues. Variables with a significant effect are shown in bold (Monte Carlo Permutation test, $p < 0.05$). -v, near village; -p near park. After Yamashita et al. (in press).

Stand Structure, Floristic Composition and Diversity of Tropical Lowland Rain Forests in Sabah, Malaysia under Different Managements

Shin-ichiro Aiba[1], Tatsuyuki Seino[2], Masaaki Takyu[3],
Kanehiro Kitayama[4], Robert C. Ong[5]
[1] *Faculty of Science, Kagoshima University, Kagoshima 890-0065, Japan*
[2] *Graduate School of Life and Environmental Sciences,*
University of Tsukuba, Nagano 384-1305, Japan
[3] *Faculty of Regional Environmental Science,*
Tokyo University of Agriculture, Tokyo 156-8502, Japan
[4] *Center for Ecological Research, Kyoto University, Ohtsu, Shiga 520-2113, Japan*
[5] *Forest Research Centre, Forestry Department, P. O. Box 1407,*
90715 Sandakan, Sabah, Malaysia

Introduction

Timber exploitation, which accelerated from the 1950s to 1970s by the introduction of mechanized methods, has been altering the tropical lowland forests of Sabah, Malaysian Borneo (Sabah Forestry Department 1989). For sustainable production of low-volume, high-quality and high-priced timbers, reduced-impact logging (RIL), a low impact logging technique of timber harvesting, was introduced in Sabah in 1989 (Lagan et al. 2007). It is believed that RIL is an adequate method for the sustainable management of tropical forests, because RIL can reduce the damages to the forests compared to the conventional logging method. However, there are relatively limited data to justify the sustainability of RIL in terms of full recovery of species composition, diversity and biomass after timber harvest (Pinard and Putz 1996; Bertault and Sist 1997; Sist and Nguyen-The 2002; Bischoff et al. 2005). Can the tropical forests after RIL approach the pre-harvest, old-growth status in the course of secondary succession? To answer this question, we compared the recovery processes of logged-over forests subjected to RIL and to a conventional method (high-impact logging) in terms of structure, floristic composition and diversity.

Materials and Methods

Eleven research plots of 0.2 ha area (100 m x 20 m or 50 m x 40 m, depending on the availability of similar topography) were established in Deramakot Forest Reserve (DFR) and the neighboring Tangkulap Forest Reserve (TFR), Sabah, Malaysian Borneo (5°N 117°E), under different forest managements (Table 1). DFR and TFR had been selectively logged in the 1950s-1980s with similar overall intensity. The minimum trunk diameter for harvesting was 60cm, but this rule does not appear to be necessarily followed. This resulted in very heterogeneous condition of remaining forests at the small spatial scale. After this period, DFR was selected as a focal site for the application of RIL in 1989, while TFR receives persistent pressure of conventional logging. In DFR, we established two plots in each of following four groups of forest management in May 2003. (1) Residual primary forest that appeared to be unaffected by logging,

(2) old-growth forest that was not logged after 1980s, (3) forests logged by RIL in 1995-1997, (4) forests logged by RIL in 1999-2000. The distinction between groups (1) and (2) was based on the proximity to logging roads or skid trails and on the presence or absence of stamps in the plot. Forests of groups (3) and (4) were identified by the management record of DFR (Lagan et al. 2007). In TFR, in May 2003, we established two plots in the forest that had been intermittently logged by the conventional method, and added one more plot in the same forest in March 2005. In the following analyses and discussion, we used three broad categories of forest management status as follows. Old-growth forests including (1) and (2), forests harvested by RIL including (3) and (4), and forests harvested by conventional logging (5).

All plots were divided into contiguous twenty 10-m x 10-m subplots. The location and altitude of the plots were measured by using a portable receiver of global positioning system (GPS III plus, Garmin, Olathe, USA). All living trees larger than 30 cm in trunk girth at 1.3 m above ground were measured first in May 2003 and re-measured in March 2005. Diameter at breast height (DBH) was calculated as girth divided by 3.14 (thus minimum DBH was 9.6 cm). Dead trees were checked at the tree census in March 2005. For comparison among the three categories of forest management, we incorporated the 2005 data to increase the sample size of the forest logged by conventional method. Buttressed or stilt-rooted trees were measured for trunk diameter at above the protrusions but not at 1.3m above the ground. Multiple trunks were separately recorded for DBH. Leaf area index (LAI), which is defined as the sum of leaf area per unit ground area, was measured at five plots using LAI-2000 Canopy Analyzer (LI-COR, Lincoln, USA) in June 2003. The five plots were PRI-1, 80s-2, RIL00-2, RIL05-1, and CV-1 (see Table 1 for the abbreviation of the plot). LAI was estimated based on ten measurements at corners of 10-m x 10-m subplots in each plot. This was repeated three times in different parts of the plot, from which the mean LAI was calculated.

Above-ground biomass (AGB, kg) of individual stem was estimated by using the following equations obtained by Brown (1997) and Chave et al. (2005) as:

$$AGB = \exp(-2.134+2.530 \ln(DBH)), \qquad (1)$$

and

$$AGB = \rho \times \exp(-1.499 + 2.148\ln(DBH) + 0.207(\ln(DBH))^2 - 0.0281(\ln(DBH))^3), \qquad (2)$$

respectively, where ρ is wood density (g cm^{-3}). The stand-level AGB density (kg m^{-2}) was derived by the summation of individual stem biomass divided by plot area. For equation (2), the use of stand-level average was recommended by Chave et al. (2005) if species-specific wood density was unavailable as in our case. Brown (1997) reported that mean wood density for 428 Asian tropical tree species was 0.57 g cm^{3}. If this value was used, biomass estimates by equation (2) was almost identical to those by equation (1). For simplicity, we reported the results based on equation (1) only.

To identify species, we collected leaves for voucher specimens, using a clipper and a catapult for tall trees. Species identification was based on the leaf specimens and bark characters. Voucher specimens were stored at the laboratory in DFR. We have not yet finished the matching of species between plots, so that floristic comparison among plots was done at the genus level. Four and 18 stems (0.3 and 1.4% of total number of stems) could not be identified to family and genus, respectively, and these were excluded from the calculation of number of taxa. Floristic diversity of each plot was evaluated by numbers of family, genus and species. Logged forests may have smaller numbers of taxa simply because stem density is reduced by logging. To allow for this, an index that represents diversity in terms of number of species relative to number of stem was calculated:

$$S = \alpha \ln (1+N/\alpha),$$

where S is the number of species, N is the number of stems, and α is a constant known as Fisher's diversity index (Fisher et al. 1943). To take into account the equitability of species abundance, Shannon's indices of diversity and evenness were also calculated (Magurran 2004). For floristic comparison among plots, detrended correspondence analysis (DCA) was conducted using relative basal area of genus in 2005, which was log (x+1) transformed before analysis (ter Braak & Smilauer 2002). The method of detrending used was by segments.

Dipterocarpaceae trees are dominant climax species in tropical lowland forest in Southeast Asia, and this family is well known as important commercial timber (Whitmore 1984). On the other hand, most of observed *Macaranga* (Euphorbiaceae) species were characterized as a gap-dependent species in regeneration (Slik et al. 2003). Their regeneration requires large canopy opening, and is abundant on disturbed soils (Whitmore 1984). Thus, we examined the dominances of the Dipterocarpaceae and *Macaranga* as indicators for disturbance.

Results

Stem density (number of stems per plot) and mean DBH was not significantly different among the three broad categories of forest management, although stem density tended to be lower in the forests harvested by conventional method than those in the other categories (Tukey multiple comparison tests, both $P > 0.05$, Fig. 1). Basal area, maximum DBH and AGB density were smaller in the forests harvested by conventional logging than in the rest ($P < 0.05$), while those of the old-growth forests and the forests harvested by RIL did not differ statistically from each other. AGB density of old-growth forests exceeded 48 kg m^{-2}, that of the forests harvested by RIL ranged from 31.8 to 48.1 kg m^{-2}, and that of the forests harvested by the conventional method from 9.6 to 28.4 kg m^{-2}. LAI was greater in old-growth forests than in the other categories, although this difference could not be tested statistically due to small sample size. DBH distribution of the forests harvested by RIL and the old-growth forests showed clearly L-shaped pattern (Fig. 2). DBH distributions of the forests harvested by conventional method showed the lack of larger trees (> 80 cm DBH) due to logging.

A total of 339 species belonging to 146 genera and 52 families (excluding unidentified stems) were found among 1333 stems ≥ 9.6 cm DBH, although the number of species may be reduced if matching of species in different plots was completed. The numbers of the observed taxa (families, genera and species) in the old-growth forests and the forests harvested by RIL were similar to each other, and were higher than those of the forests harvested by conventional method (Tukey multiple comparison tests, all $P < 0.05$; Fig. 3). The index of species richness of Fisher's α in the old-growth forests and the forests harvested by RIL was higher than that in the forests harvested by the conventional method (all $P < 0.05$). The α of the old-growth forests and the forests harvested by RIL overlapped greatly, all being greater than 50, while that of the forests harvested by conventional method was less than 40. The maximum value of α was 119.3 in the old-growth forest at PRI-1 and the minimum value was 17.6 in the forest harvested by conventional method at CV-3. Both of Shannon's indices of species diversity and evenness showed similar trends as number of taxa and Fisher's α̃.

The first and second axes of DCA cumulatively explained 24.4% of variance (eigenvalues, 0.34 and 0.21, respectively). Floristics of the three forest categories evaluated by genus composition clearly differentiated along the axis 1 (Fig. 4). The ranges of axis 1 scores of old-growth forests and the forests harvested by RIL partly overlapped, but the forests harvested by conventional logging were separated from the other two categories. Therefore, a most striking difference in species composition was found between the forests harvested by the conventional method and the rest. Among four abundant genera, two (*Dipterocarpus* and *Shorea*) that belong to Dipterocarpaceae were biased towards old-growth forests. Two genera of Euphorbiaceae showed contrasting patterns: *Macaranga* were abundant in disturbed sites while *Mallotus* were so in well-developed forests.

Old-growth forests and the forests harvested by RIL were dominated by Dipterocarpaceae trees, while the forests harvested by conventional method were by *Macaranga* trees (Fig. 5). According to the pattern of DBH distribution, Dipterocarpaceae contained many small-diameter trees in both the old-growth forests and the forests harvested by RIL, indicating a good regeneration (Fig. 2). On the other hand, *Macaranga* trees regenerated well in the forests harvested by the conventional method.

Discussion

Our results suggest that RIL is an efficient method to reduce logging impacts on forest structure, floristic composition and diversity compared with the conventional logging method. Commercially-logged forests occupy large tracts in tropical regions where human impacts are becoming increasingly greater, yet they have a high potential to protect biodiversity if managed properly (Cannon et al. 1998). The size of canopy opening by logging (i.e. creation of canopy gap) affected regeneration patterns and species composition elsewhere (Denslow 1980; Pickett and White 1985). RIL operation regulates the amount of logged trees and their size (DBH), location, and transportation of harvested logs (Sabah Forestry Department and European Union 2000). Consequently, RIL operation creates a smaller number of canopy gaps probably with a smaller mean size than the conventional logging does. Shade-tolerant trees can regenerate under a darker light condition. Therefore, these trees could have regenerated under small-sized canopy gaps in the forests harvested by RIL due to their physiological tolerance for reduced light. In contrast, the regeneration of shade-intolerant trees requires a sunnier condition (Turner 2001). Response to light condition associated with the difference in gap sizes caused a greater similarity of species composition and diversity between the old-growth forests and the forests harvested by RIL, and facilitated the regeneration of dipterocarp trees in the forests harvested by RIL (Sist and Nguyen-The 2002; Bischoff et al. 2005).

Differences of forest managements were related to the differences in the disturbance regime and regeneration patterns. For example, bulldozers heavily disturb topsoils by pulling out logged trees on the forest floor in the forests harvested by conventional method (Pinard et al. 2000). In our study, the forests harvested by conventional method were dominated by pioneer species of the genus *Macaranga*. *Macaranga* trees are known to regenerate under large canopy gaps often with disturbed soil conditions (Davies et al. 1998; Davies 2001). Moreover, an operation of the conventional method would have left a greater damage to the surrounding trees. On the other hand, RIL operation is gentle to forest with a minimum damage to soils and surrounding trees. This was achieved by both pre-harvest and harvest operations, including careful planning and construction of skid trails or skylines, directional felling, and appropriate skidding and landing (Sabah Forestry Department and European Union 2000). Our analysis dealt with two to three decades of a secondary succession only, and whether the species composition and structure of the forest including shrubs and herbs (that we do not include in our current analysis) can fully recover to a pre-harvest condition is still not known. To confirm the sustainability of the structure and floristic composition of tropical rain forests in DFR managed by RIL, long-term ecological monitoring is needed.

References

Bertault JG, Sist P (1997) An experiment comparison of different harvesting intensities with reduced-impact and conventional logging in East Kalimantan, Indonesia. For Ecol Manage 94:209-218

Bischoff W, Newbery DM, Lingenfelder M, Schnaeckel R, Petol GH, Madani L, Ridsdale CE (2005) Secondary succession and dipterocarp recruitment in Bornean rain forest after logging. For Ecol Manage 218:174-192

Brown S (1997) Estimating biomass and biomass change of tropical forests. A primer. FAO, Rome.

Cannon CH, Peart DR, Leighton, M (1998) Tree species diversity in commercially logged Bornean rainforest. Science 281:1366-1368.

Chave J, Andalo C, Brown S, Cairns MA, Chambers JQ, Eamus D, Folster H, Fromard F, Higuchi N, Kira K, Lescure JP, Nelson BW, Ogawa H, Puig H, Riera B, and Yamakura T. (2005) Tree allometry and improved estimation of carbon stocks and balance in tropical forests. Oecologia 145:87-99.

Davies SJ (2001) Tree mortality and growth in 11 sympatric Macaranga species in Borneo. Ecology 82:920-932

Davies SJ, Palmiotto PA, Ashton PS, Lee HS, Lafrankie JV (1998) Comparative ecology of 11 sympatric species of Macaranga in Borneo: tree distribution in relation to horizontal and vertical resource heterogeneity. J Ecol 86:662-673

Denslow JS (1980) Patterns of plant species diversity during succession under different disturbance. Oecologia 46: 18-21

Fisher RA, Corbert AS, Williams CB (1943) The relation between the number of species and the number of individuals in a random sample of an animal population. J Animal Ecol 12:42-58

Lagan P, Mannan S, Matsubayashi H (2007) Sustainable use of tropical forests by reduced-impact logging in Deramakot Forest Reserve, Sabah, Malaysia. Ecol Res 22:414-421.

Magurran AE (2004) Measuring biological diversity. Blackwell, Malden

Pickett STA, White PS (1985) The ecology of natural disturbance and patch dynamics. Academic Press, Orland

Pinard MA, Putz FE (1996) Retaining forest biomass by reducing logging damage. Biotropica 28:278-295

Pinard MA, Barker MG, Tay J (2000) Soil disturbance and post-logging forest recovery on bulldozer paths in Sabah, Malaysia. For Ecol Manage 130:213-225

Sabah Forestry Department (1989) Forestry in Sabah. Sabah Forestry Department, Sandakan

Sabah Forestry Department and European Union (2000) RIL: operation guide book, specifically for crawler tractor use, 2nd ed. Sabah Forestry Department and European Union, Sandakan and Brussels

Sist P, Nguyen-The N (2002) Logging damage and the subsequent dynamics of a dipterocarp forest in East Kalimantan (1990-1996). For Ecol Mange 165:85-103

Slik JWF, Kessler PJA, van Welzen PC (2003) *Macaranga* and *Mallotus* species (Euphorbiaceae) as indicators for disturbance in the mixed lowland dipterocarp forest of East Kalimantan (Indonesia). Ecol Indicators

2:311-324

ter Braak CJF, Smilauer P (2002) CANOCO reference manual and CanoDraw for Windows user’s guide: software for canonical community ordination (version 4.5). Microcomputer Power, Ithaca

Turner IM (2001) The ecology of trees in the tropical rain forest. Cambridge University Press, Cambridge

Whitmore TC (1984) Tropical rain forests of the Far East. Second Edition. Oxford University Press, Oxford

Table 1. Description of the research plots.

Plot name	Abbreviation	Plot size (m)	Altitude (m)	Management status
Old-growth unlogged since the 1980s				
Ecological trail	PRI-1	100 x 20	248	Primary forest
ET antenna	PRI-2	50 x 40	248	Primary forest
ET jauh	80s-1	50 x 40	248	Unlogged since the 1980s
C54	80s-2	50 x 40	195	Unlogged since the 1980s
RIL				
Mannan	RIL95-1	100 x 20	196	8-10 years after RIL
Domingo	RIL95-2	100 x 20	200	8-10 years after RIL
C63 bawah	RIL00-1	100 x 20	195	5-6 years after RIL
C63 atas	RIL00-2	100 x 20	221	5-6 years after RIL
Conventional method				
Tangkulap 1	CV-1	100 x 20	109	Conventional logging
Tangkulap 2	CV-2	50 x 40	76	Conventional logging
Tangkulap 3	CV-3	100 x 20	52	Conventional logging

RIL indicates reduced-impact logging, and the time after logging is as of 2005.

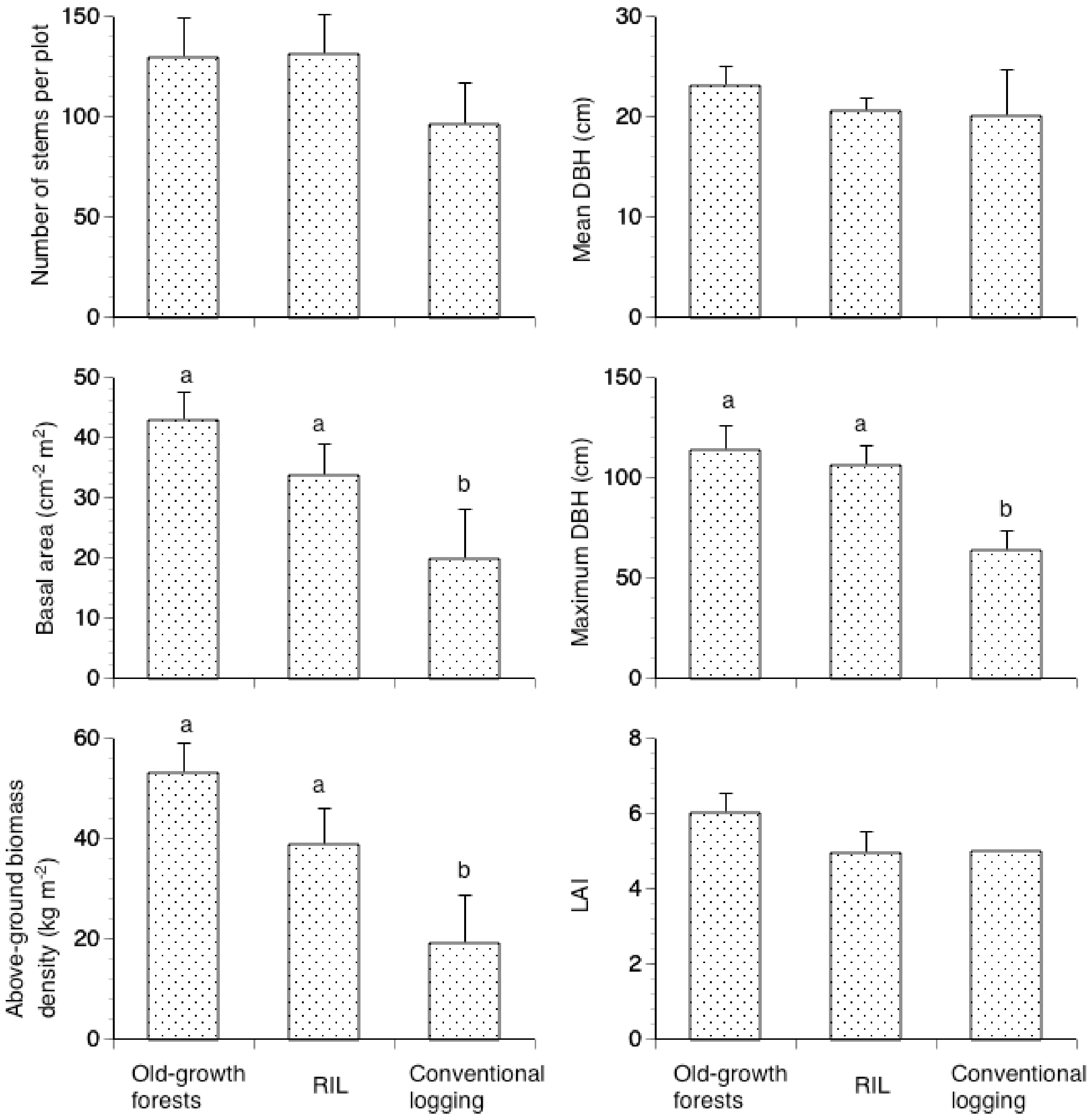


Figure 1. Stand structure in relation to management status in the tropical lowland forest in Deramakot, Sabah, Malaysia. RIL and conventional logging indicate the forests harvested by reduced-impact logging and by conventional logging, respectively. Forest categories that do not share the same letters differ at $P < 0.05$ by Tukey multiple comparison test. Vertical lines indicate standard deviation.

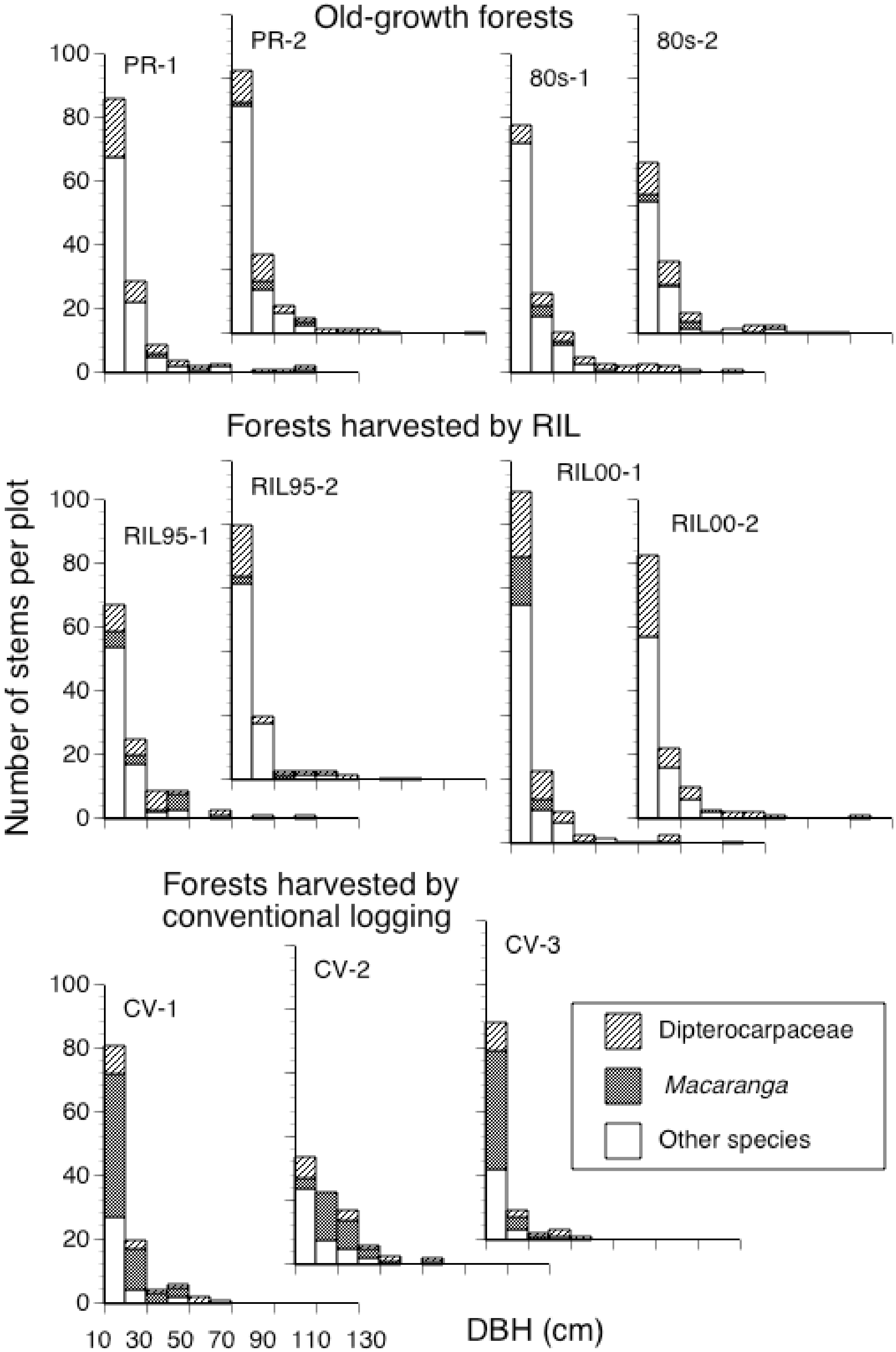


Figure 2. DBH distribution of trees in the 11 study plots, showing the fractions occupied by two dominant taxa, Dipterocarpacea and *Macaranga* (Euphorbiaceae). Stems < 10 cm DBH were excluded. See Table 1 for the plot abbreviations.

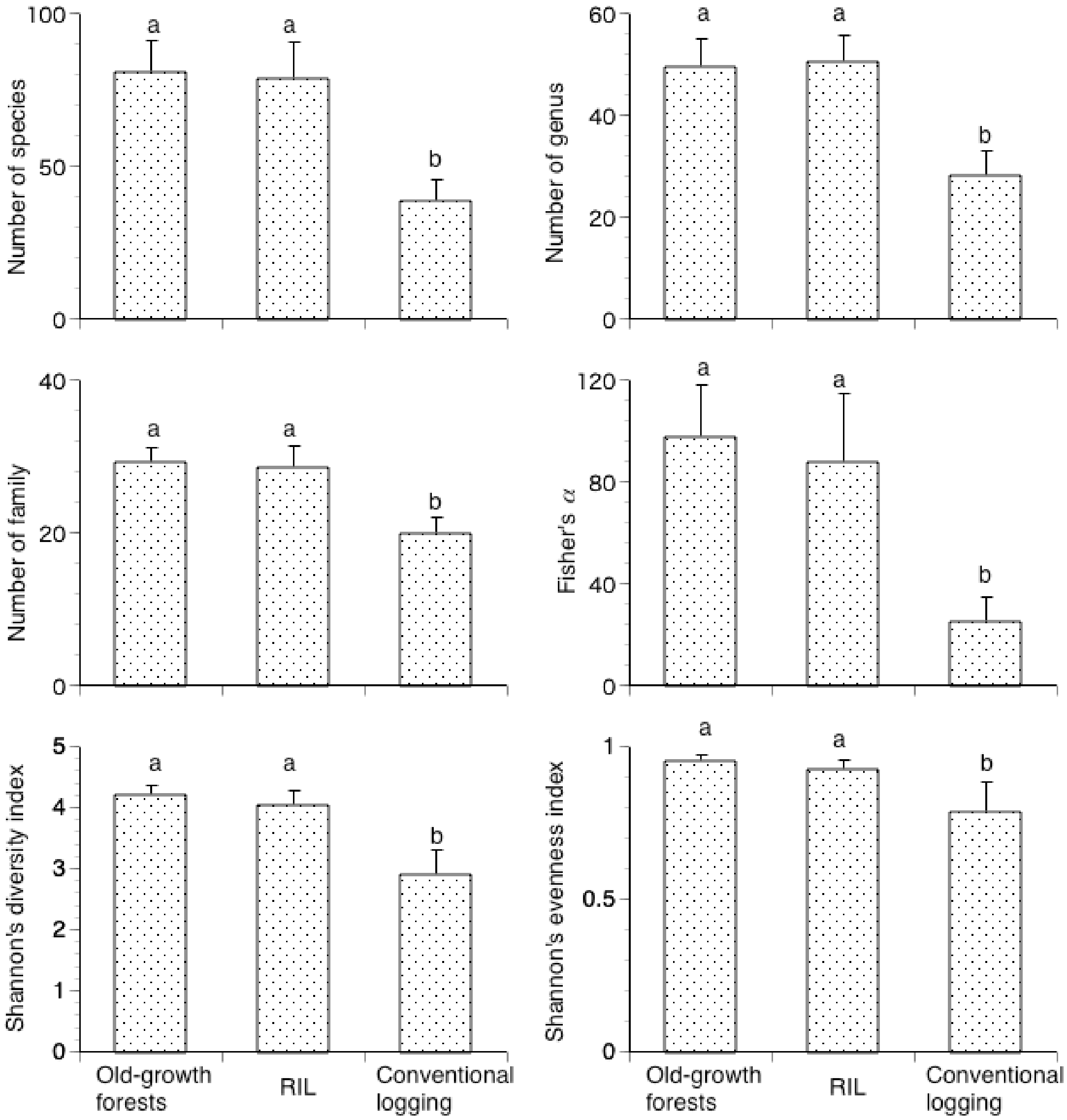


Figure 3. Tree diversity in relation to management status in the tropical lowland forest in Deramakot, Sabah, Malaysia. RIL and conventional logging indicate the forests harvested by reduced-impact logging and by conventional logging, respectively. Forest categories that do not share the same letters differ at $P < 0.05$ by Tukey multiple comparison test. Vertical lines indicate standard deviation.

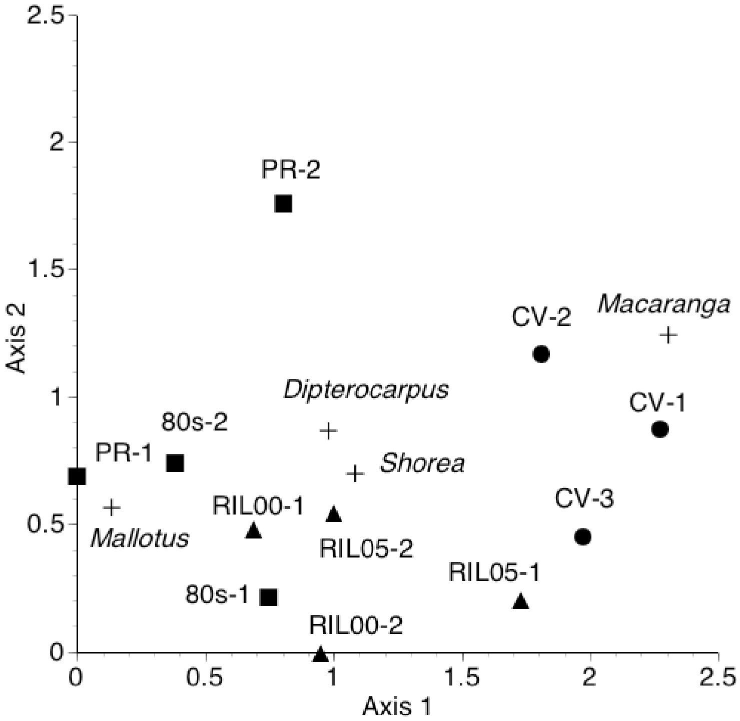


Figure 4. DCA diagram based on relative basal area of genus. Scores of four abundant genera were also shown. Square, old-growth forests; triangle, forests harvested by reduced-impact logging; circle, forests harvested by conventional method; cross, genus. See Table 1 for the plot abbreviations.

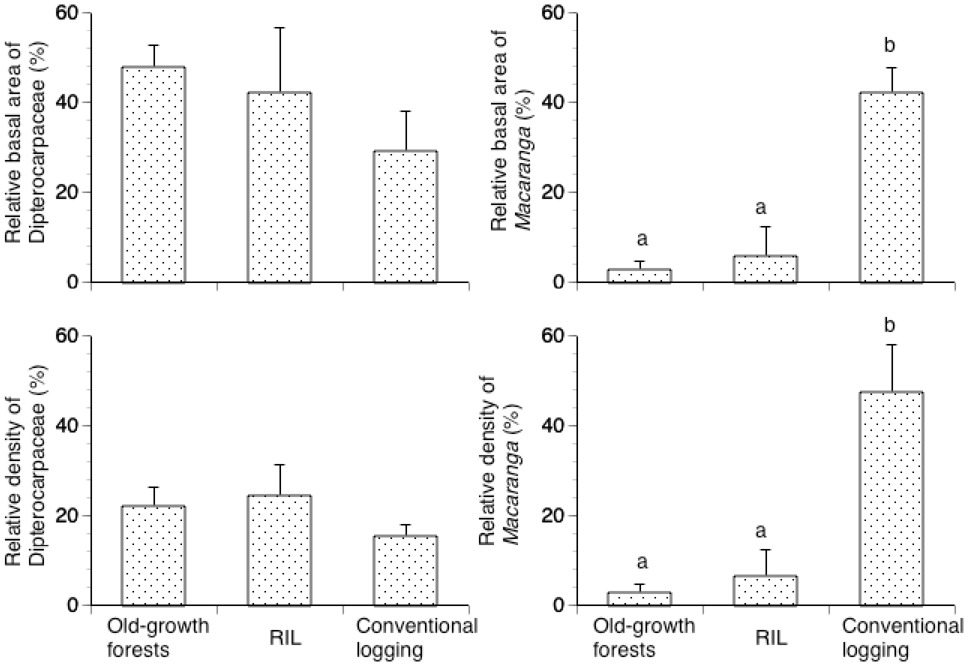


Figure 5. Dominance of Dipterocarpaceae and *Macaranga* (Euphorbiaceae) in terms of basal area and stem number in the three categories of forest management. RIL and conventional logging indicate the forests harvested by reduced-impact logging and by conventional logging, respectively. Forest categories that do not share the same letters differ at $P < 0.05$ by Tukey multiple comparison test. Vertical lines indicate standard deviation.

Effects of Logging on Forest Structure and Drosophilid Diversity in Tropical Lowland Forests of Sabah, Malaysia

Kosuke Akutsu[1], Khen V. Chey[2], Masanori J. Toda[1]
[1]*Institute of Low Temperature Science, Hokkaido University,*
Kita 19 Nishi 8,Kitaku, Sapporo, Hokkaido, 060-0819 Japan
[2]*Forest Research Center, Forestry Department, Sandakan, Sabah,*
Malaysia,P. O. Box 1407, 90715 Sandakan, Sabah, Malaysia

Introduction

Tropical forests are the most diverse and ecologically complex of the world's ecosystems and their disappearance has been a major issue in conservation ecology since the 1980s (Bowles et al. 1998). The importance of both ecosystem services and the biological value of tropical forests has been emphasized by scientists and has caught the interest of citizens throughout the world. Sustaining biodiversity in tropical forests is a primary objective of conservation ecology. In addition, understanding the mechanisms of biodiversity degradation caused by logging disturbances in tropical forests is an important area of research in community ecology (Widodo et al. 2004).

In tropical forests that have regenerated after clearcut or that have been converted to plantations, insect diversity decreases from pre-disturbance levels (Eggleton et al. 1996). However, the effects of selective logging (i.e., a relatively moderate disturbance) on insect diversity remain unclear (Eggleton et al. 1996). In Sabah, Malaysia, a recently adopted logging method, i.e., reduced impact logging (RIL), has been launched to maintain sustainable forest use with minimal forest disturbance (Lagan et al. 2007). RIL was designed to limit forest disturbance and conserve large commercial trees as potential seed sources. Nevertheless, logging operations cause distinct changes in forest structure such as canopy destabilization and decreases in the density of trees or saplings (Hall et al. 2003).

The vertical distribution of the drosophilid community is closely related to the vertical foliage structure (Toda 1992; Tanabe et al. 2001). Specifically, drosophilid diversity tends to be higher in vertically complex than in simple forests (Tanabe et al. 2001). This difference in diversity can be explained in part by the "habitat heterogeneity hypothesis" whereby structurally complex habitats may provide more niches and more diverse ways of exploiting environmental resources and may thus increase species diversity (MacArthur and MacArthur 1961). Furthermore, drosophilid flies are useful indicators of biodiversity because their short lifespan (approximately 1 month) promotes rapid responses of their populations to environmental changes. In addition, their mobility allows quick relocation from unfavorable to favorable habitats (van der Linde and Sevenster 2002).

To test the habitat heterogeneity hypothesis, we investigated the effects of forest structural changes caused by various logging intensities on the species diversity of the drosophilid community. In addition, we discuss the relationship between biodiversity conservation and forest use by humans.

Material & Methods

Study plots

To assess the effects of various logging intensities on forest structure and the drosophilid community, we chose five study plots that experienced different disturbance regimes in the Deramakot Forest Reserve (DFR), Sabah, Malaysia. The disturbance regimes were characterized by two factors: the logging method [RIL or conventional (i.e., non-RIL) methods (CV)] and the time elapsed after logging. The five plots were differentiated according to the disturbance regimes (Table 1): “PRI” (primary forest with no logging), “80s” (harvested in the 1980s by CV), “RIL95” (harvested in 1995 by RIL), “RIL00” (harvested in 2000 by RIL), and “CV” (harvested continuously by CV).

Forest structure

In March 2005, the three-dimensional forest structure was measured using a portable lidar system. Details of the system were described by Parker et al. (2004). To measure vertical forest profiles, each plot was separated into lattices of each square 10-m on a side. Measurements were conducted at 2-m intervals along each separated line. For each 10-m section, vertical foliage densities were calculated at 1-m height intervals using the MacArthur–Horn method (MacArthur and Horn 1969) by combining data (i.e., height differences between the lidar system and the nearest foliage) from five measurement points.

Insect sampling

We used a bait (fermented banana) trap designed specifically for collecting drosophilid flies (Toda 1977). We selected 1–2 trees adjacent to each plot (within 5 m) for the placement of traps. Traps were set vertically from the understory to the canopy at each trapping site, with the lowest trap set at 0.5 m above the ground surface. The next trap was placed at 1.5 m aboveground, and remaining traps were set at 5-m intervals up into the canopy. The top traps varied in height depending on the canopy height of the forest (Table 1). Several (up to four) upper traps were suspended from the same rope using a pulley that was hung from a branch of the selected tree, and the lowest two traps were tied directly to the trunk of the same or a nearby tree (Table 1). We sampled four times in July–August and October–November 2003, and January–February and April–May 2004. During each sampling period, trapped insects were collected and trap baits were renewed three times at 10-day intervals. Samples from the most productive (determined from the sum of drosophilid flies across all plots) 10-day period during each season were selected for analyses. Flies were identified to species or morphospecies of Drosophilidae.

Data analyses

Forest structure

To estimate the vertical complexity of foliage, the foliage height diversity (FHD) was measured using the Shannon–Wiener index (Tanabe et al. 2001) for each 10-m section, and the mean FHD was calculated for each plot. Because of differences in plot shape (Table 1) and limited access caused by large fallen trees, the number of 10-m sections differed among plots (30 at PRI, RIL95, and RIL00; 24 at 80s; and 29 at CV). As an additional measure of foliage structure, the horizontal variance of the vertical foliage profile (HVF) was calculated as follows: the coefficient of variance for foliage densities at each 1-m height interval across all

10-m sections within each plot was calculated, and then the coefficients were averaged for all 1-m height intervals. Heights with no foliage across all 10-m sections were omitted from the calculations.

Differences in FHD or HVF were tested using one-way ANOVA. When a significant difference was detected among plots, multiple comparisons were performed using Tukey's honestly significant difference (HSD) test.

Diversity of the drosophilid community

The species richness and several diversity indices were calculated for each plot during each sampling period. Because the number of collected individuals and thus the number of species may have been biased by the number of traps, which varied among plots, species richness was estimated using sample rarefaction (Heck et al. 1975), based on the plot containing the lowest number of individuals in each season. Alpha, beta, and gamma diversity were calculated according to the concept of "additive partitioning of species diversity"; i.e., gamma (total) diversity can be partitioned into alpha and beta diversity (Levins 1968, Tanabe et al. 2001, Veech et al. 2002). Gamma diversity was calculated using the Shannon-Wiener formula, based on summed data for all traps in each plot. Alpha (intra-trap) diversity was estimated as the average Shannon-Wiener diversity calculated for each trap. Beta (inter-trap) diversity caused by species' compositional differences among traps was calculated as the difference between gamma and alpha diversity (gamma – alpha). As a measure of equitability, interspecific variation in the number of individuals was measured using Pielou's *J'*, based on gamma diversity and the observed number of species in each plot.

Generalized linear models in R (R Development Core Team 2004) were used to estimate the effects of forest structure. Separate analyses were conducted for species richness and each diversity index of the drosophilid community. Species richness was assumed to be Poisson distributed, whereas the diversity indices were assumed to be Gaussian distributed. The link functions were set as logarithmic. In addition, the four sampling periods were incorporated into the analyses as a categorical variable (the sampling period July–August was incorporated into the intercept). From all possible model combinations (including the null model with no variables) that incorporated the covariates (FHD and HVF) and one cofactor (season), the best model was selected using the Akaike information criterion (AIC; Akaike 1973). The deviance improvement was evaluated for the selected model. The percentage improvement compared to the deviance of the null model was used to estimate the relative explanatory power of the selected model.

Results

Forest structure

FHD was highest in the undisturbed plot of PRI and lowest in the most disturbed plot of CV. The moderately disturbed plots (80s, RIL00, and RIL95) were intermediate in FHD, between PRI and CV (Fig. 1). FHD differed significantly among plots (ANOVA, $P < 0.01$). PRI was significantly different from all the other plots (Tukey HSD, $P < 0.01$), but no other plots were significantly different from each other. Thus, all logging operations (even RIL) clearly affected forest structure by reducing FHD.

The mean HVF also differed significantly among plots (ANOVA, $P = 0.014$). This was primarily driven by the difference between PRI and RIL95 (Tukey HSD, $P = 0.039$; Fig. 1). HVF tended to be higher in the moderately disturbed plots.

Changes in drosophilid diversity with respect to forest structure and season

In total, 20883 individuals representing 80 species of drosophilid fly were identified (PRI: 5149 individuals, 52 species; 80s: 4348, 45; RIL00: 4398, 49; RIL95: 4344, 44; CV: 2644, 45). All of the diversity measures, with the exception of equitability, fluctuated significantly among seasons (Fig. 2). In particular, the variation in species richness was largely explained by season (76.07% deviance improvement; Table 2). The number of species, gamma diversity, and alpha diversity were lowest in May and highest in January. Throughout the four seasons at DFR, the most rainy days occurred in December–January, whereas the least rain fell in April–May. Because most drosophilid species are vulnerable to desiccation, moisture conditions are a key factor for drosophilid survival. Of the two measures of forest structure, only FHD positively affected beta diversity (Table 2). Equitability was not affected by either season or forest structure.

Discussion

Effects of logging disturbances on forest structure

Logging disturbances decreased the vertical heterogeneity of foliage density. Logging in DFR targets mature Dipterocarpaceae trees (diameter at breast height > ca. 60 cm) and has removed several large foliage crowns from the canopy and/or emergent layers. These losses of canopy foliage reduce the complexity of the vertical foliage structure and promote the growth of subcanopy and/or pioneer trees (Okuda et al. 2003). As this gap regeneration proceeds, the vertical foliage structure slowly recovers. However, our results indicate that the effects of logging remain prevalent in the vertical foliage structure of forests harvested by both conventional logging as early as the 1980s and by RIL in 1995.

In contrast, the horizontal heterogeneity of foliage density tended to be higher in moderately disturbed forests. According to theories addressing light acquisition by subcanopy trees, the foliage distribution is predicted to be stratified in mature forests with a closed canopy (Terborgh 1985), thus forming a horizontally continuous structure of dense and sparse foliage layers. The selective removal of large trees from the canopy layer and the subsequent gap regeneration may disturb this horizontal constancy of the foliage structure. However, at the most strongly disturbed plot (CV), the horizontal foliage heterogeneity was relatively low. This may be attributed to the disappearance of virtually all trees in the top layer by heavy logging, as well as the concomitant growth of subcanopy and pioneer trees.

Relationship between drosophilid diversity and forest structure

Species richness was independent of forest structure and fluctuated significantly among the four seasons. The stability of equitability throughout the seasons suggests that seasonal changes in gamma and alpha diversity were caused by seasonal changes in species richness. In contrast, beta diversity (representing the degree of vertical habitat segregation in the drosophilid community) was positively affected by FHD. These results are consistent with the habitat heterogeneity hypothesis, which predicts that structurally complex habitats usually accommodate more species than homogeneous habitats by providing a greater variety of microhabitats. Paradoxically, in forests with less obviously stratified standing trees, weakened habitat segregation may strengthen resource competition within the drosophilid community if component species use similar food resources (Shorrocks 1977; Tanabe et al. 2001). This strengthened competition may accelerate the extinction of species and may ultimately decrease local species richness.

In DFR, decreases in FHD caused by forest logging were related to decreases in beta diversity, but not to changes in species richness. Selective logging, especially RIL, in DFR has been operated to minimize the widespread effects of logging, thus reserving forest stands with diverse mosaics of foliage structure. Although species richness may decrease through strengthened competition in poorly stratified forest stands, adjacent stands with sufficient foliage stratification may function as regional sources of species to compensate for the local depletion of species richness. This seeding effect would be especially strong for highly mobile organisms such as flying insects and may thus serve as a reason why drosophilid species richness did not significantly vary among the study plots that differed in vertical foliage structure.

From a conservation ecology standpoint, we conclude that logging operations in DFR appear to be successful in terms of the conservation of drosophilid biodiversity. However, we caution against the generalization of our results to other organisms that may vary in mobility and in their responses to different sizes of habitat patchiness.

Acknowledgements

We thank the staff of DFR and the Forest Research Centre, Sabah, Malaysia, particularly, Peter Lagan, Azny Ahmad, and Erwin for their kind support in the field.

References

Akaike H (1973) Information theory as an extension of the maximum likelihood principle. In: Proceedings of the second international symposium on information theory. Budapest, Hungary, pp. 267-281

Bowles IA, Rice RE, Mittermeier RA, da Fonseca GAB (1998) Logging and tropical forest conservation. Science 280: 1899-1900

Eggleton P, Bignell DE, Sands WA (1996) The diversity, abundance and biomass of termites under differing levels of disturbance in the Mbalmayo Forest Reserve, southern Cameroon. Philos Trans R Soc London, Ser B 351: 51-68

Hall JS, Harris DJ, Medjibe V, Ashton PMS (2003) The effects of selective logging on forest structure and tree species composition in a Central African forest: implications for management of conservation areas. For Ecol Manage 183: 249-264

Heck KLJ, van Belle G, Simberloff D (1975) Explicit calculation of the rarefaction diversity measurement and the determination of sufficient sample size. Ecology 56: 1459-1461

van der Linde K, Sevenster JG (2002) Drosophila diversity over a disturbance gradient. Proceedings Experimental and Applied Entomology 13: 51-56

Lagan P, Mannan S, Matsubayashi H (2007) Sustainable use of tropical forests by reduced-impact logging in Deramakot Forest Reserve, Sabah, Malaysia. Ecol Res 22: 414-421

Levins R (1968) Evolution in changing environments, some theoretical explorations. Princeton University Press, Princeton

MacArthur RH, Horn H (1969) Foliage profile by vertical measurements. Ecology 50: 802-804

MacArthur RH, MacArthur JW (1961) On bird species diversity. Ecology 42: 594-598

Okuda T, Suzuki M, Adachi N, Quah ES, Hussein NA, Manokaran N (2003) Effect of selective logging on canopy and stand structure and tree species composition in a lowland dipterocarp forest in peninsular Malaysia. For Ecol Manage 175: 297-320

Parker GG, Harding DJ, Berger ML (2004) A portable LIDAR system for rapid determination of forest canopy structure. J Appl Ecol 41: 755-767

R Development Core Team (2004) R: a language and environment for statistical computing.

http://www.R-project.org (R Foundation for Statistical Computing: Vienna Austria.)

Shorrocks B (1977) The distribution and abundance of woodland species of British *Drosophila* (Diptera: Drosophilidae). J Anim Ecol 44: 851-864

Tanabe S, Toda MJ, Vinokurova AV (2001) Tree shape, forest structure and diversity of drosophilid community: Comparison between boreal and temperate birch forests. Ecol Res 16: 369-385

Terborgh J (1985) The vertical component of plant species diversity in temperate and tropical forests. Am Nat 126: 760-776

Toda MJ (1977) Two new 'retainer' bait traps. Drosophila Inf Serv 52: 180

Toda MJ (1992) Three-dimensional dispersion of drosophilid flies in a cool temperate forest of northern Japan. Ecol Res 7: 283-295

Veech JA, Summerville KS, Crist TO, Gering JC (2002) The additive partitioning of species diversity: recent revival of an old idea. Oikos 99: 3-9

Widodo ES, Naito T, Mohamed M, Hashimoto Y (2004) Effects of selective logging on the arboreal ants of a Bornean rainforest. Entomol Sci 7: 341-349

Table 1 The disturbance regime, shape, canopy height, and trapping profile of each study plot.

Plot name	Abbreve	Disturbance regime	Plot size (m x m)	Canopy height (m)	Tree species at trap-sites	Trap heights (m)
Ecological trail	PRI	No impact of logging	20 x100	31.5-36.5	*Polygclaccae affine* *Shorea exelliptica*	0.5, 1.5, 6.5, 11.5, 16.5, 21.5, 26.5, 31.5, 36.5
C54	80s	Harvested in the 1980s by CV	40 x 50	26.5-31.5	*Lithocarpus* sp. *Shorea macroptera*	0.5, 1.5, 6.5, 11.5, 16.5, 21.5, 26.5, 31.5
C63 bawah	RIL00	Harvested in 1995 by RIL	20 x 100	26.5-31.5	*Shorea* sp.	0.5, 1.5, 6.5, 11.5, 16.5, 21.5, 26.5
Mannan	RIL95	Harvested in 2000 by RIL	20 x 100	26.5-31.5	*Dipterocarpus* sp. *Dorio* sp.	0.5, 1.5, 6.5, 11.5, 16.5, 21.5, 26.5
Tangkulap 1	CV	Continued intermittent harvest by CV	20 x 100	21.5-26.5	*Shorea parviforia*	0.5, 1.5, 6.5, 11.5, 16.5, 21.5

Abbrev; Abbreviation

Table 2 Parameter estimates and percentage deviance improvement of the selected models explaining the spatio-temporal variation in species richness and diversity indices of the drosophilid community.

	Intercept		FHD		HVF		January		May		October		(%)$^{\#}$
	Est	SE	Est	SE	Est	SE	Est	SE	Est	SE	Est	SE	
Species richness	2.986	0.100	NS*		NS		0.408	0.130	-0.238	0.151	0.159	0.139	76.07
Equitability	-0.560	0.039	NS		NS		NS		NS		NS		0.00
Gamma diversity	0.597	0.077	NS		NS		0.180	0.100	-0.203	0.122	0.073	0.105	44.60
Alpha diversity	0.408	0.084	NS		NS		0.181	0.110	-0.230	0.135	-0.027	0.121	41.93
Beta diversity	-2.721	0.769	0.646	0.309	NS		0.217	0.214	-0.054	0.242	0.455	0.197	46.37

Est: Estimate, *NS: not selected, $^{\#}$;Deviance improvement

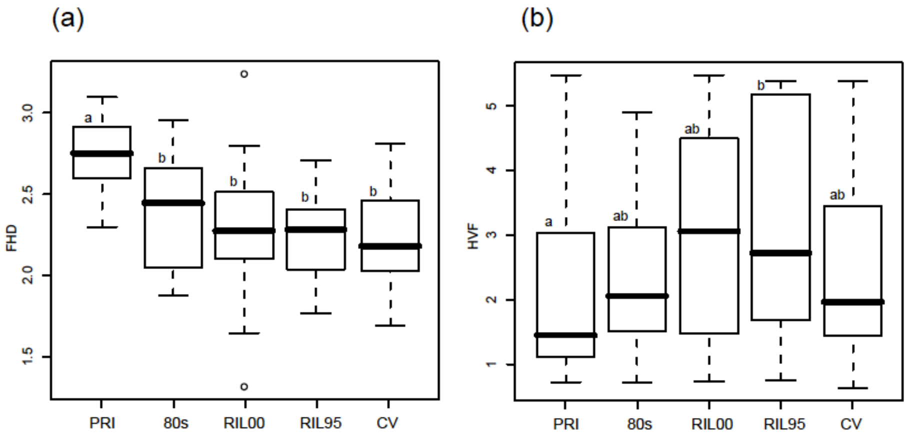


Fig. 1 Box-plots indicating the median (bold solid line), lower and upper hinges, extreme lower and upper whiskers, and outliers of (a) foliage height diversity (FHD) and (b) horizontal variance of foliage (HVF) within each plot. Different letters indicate statistically significant differences ($P < 0.05$) according to Tukey's HSD.

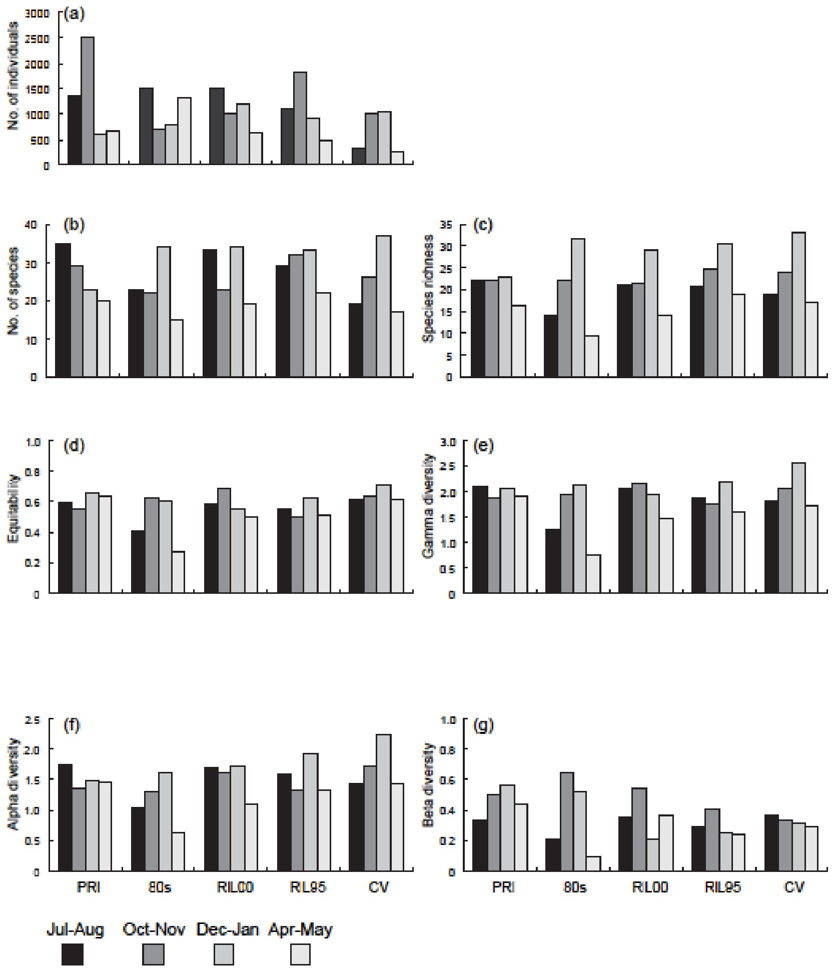


Fig. 2 Seasonal and among-plot variation in the (a) number of collected individuals, (b) number of collected species, (c) species richness estimated by rarefaction, (d) equitability, (e) gamma diversity, (f) alpha diversity, and (g) beta diversity.

Logging Effects on Soil Macro- and Meso-Faunas in the Rain Forests of Deramakot Forest Reserve, Sabah, Malaysia

Masamichi T. Ito[1], Motohiro Hasegawa[2], Tatsuya Kawaguchi[1]

[1]*Faculty of Environment and Information Sciences, Yokohama National University,*
79-7 Tokiwadai, Hodogaya, Yokohama, Kanagawa 240-8501, Japan
[2]*Kiso Experimental Station, Forestry and Forest Products Research Institute,*
Fukushima 5473, Kiso, Kisogun, Nagano 397-000, Japan

Introduction

Soil animals play important roles in every forest ecosystem in the world. Their abundance and diversity are considered to be a good indicator of forest healthiness. In Borneo, the effects of forest management have been investigated on moths (Chey 4et al. 1997), canopy arthropods (Chey et al. 1998), butterflies (Wilott et al. 2000) and beetles (Chung et al. 2000), but less frequently documented on soil animals except for termites (Eggleton et al. 1999). On the other hand, many studies which were carried out in the temperate zones of Europe, North America and East Asia revealed that forest clear-cutting caused substantial changes in soil fauna.

The purpose of our study is to detect the effects of different forest management schemes on soil fauna in tropical rain forests and to ultimately recommend a sound scheme for biodiversity conservation there.

Materials and Methods

Study sites

Ten sites were selected from forest stands under different management schemes in and around Deramakot Forest Reserve (DFR). The ten sites were grouped into three categories of management schemes: “unlogged” category including two primary-forest (PRI) sites and two sites which experienced a modest harvest by selective logging in the 1980s (80s), “RIL (reduced-impact logging)” category including two sites which were harvested by RIL in 1995 (RIL95) and two sites which were harvested by RIL in 2000 (RIL00), and “CV (conventional)” category including two sites which were continuously harvested by conventional selective logging until the time of our analysis.

Soil macrofauna

Sampling of soil macrofauna was conducted at five quadrats (each 25 × 25 cm) set at 10 m intervals along a line (40 m) in each site. Litter layer and topsoil (15 cm deep) were collected at each quadrat. The weight of litter layer and the water content were measured by drying the samples. Soil animals were immediately picked up from the soil and litter by hands in the field and preserved in 80 % ethanol. They were sorted to the groups listed in Table 1 under a stereo-microscope in the laboratory, and the sorted taxonomic groups were classified into four functional groups. Lavelle et al. (1995) recognized two important functional groups, ecosystem engineers and litter transformers. The former develops mutualism with internal microorganisms and can digest litter directly. Therefore, they affect nutrient cycling and/or soil formation, and are important in ecosystem functioning. Earthworms and termites are typical ecosystem engineers. Litter transformers such as Isopoda, Diplopoda, Blattodea and Diptera contribute to the decomposition of litter in association with external microorganisms. Predators such as Araneae, Pseudoscorpiones, Opiliones, Geophilomorpha, Symphylla and Lithobiomorpha have some roles in structuring soil animal communities. Ants act in various ways for ecosystem functioning, some as predators, others as decomposers and so on. In this study, we treated them together as one category "ants" because of the lack of information on their ecology.

Soil mesofauna

A 100 ml soil sample (20 cm^2 × 5 cm in depth) including litter layer was taken by a cylindrical core sampler at each of the ten points set at 5 m intervals along another line (45 m) in each site. The samples were put on Tullgren funnels within a few hours after sampling. Soil mesofauna was extracted for three days under irradiation of 40W electric bulbs and preserved in 80% ethanol. Among them, Collembola and oribatid mites were identified to specific level and counted under a microscope in the laboratory. Collembolan species were classified into three feeding groups, detritus feeders, fungal feeders and sucking feeders (Hasegawa 2006).

Statistical analyses

The variations in soil animal density among the forest management schemes and among the sites of each scheme were analysed by two-level nested ANOVA (group = management category, subgroup = site), separately for the total macrofauna, each functional group of macrofauna and each feeding group of Collembola. Canonical correspondence analysis (CCA) was used to examine the relation between environmental variables and the composition of the taxonomic groups of macrofauna in the study sites (ter Braak 1986). The following environmental variables were used in the analysis: the attributes based on vegetation data for all trees with DBH (maximum diameter at breast height) ≥10 cm were tree density, total basal area, above-ground biomass, the number of tree families, the number of tree species, Shannon-Wiener's diversity index, Fisher's alpha diversity index, the basal area percentage of Dipterocarpaceae, the basal area percentage of Euphorbiaceae, and the basal area percentage of Macaranga (Seino et al. 2006); soil environmental variables were the weight of organic matter and the water content in litter layer. Among them, significant variables ($p < 0.05$) were chosen by the forward selection.

Results and Discussion

Soil macrofauna

The mean densities of soil macrofauna in each of the ten sites are shown in Table 1. The mean density of total soil macrofauna was significantly higher in the unlogged sites than in the RIL sites ($p<0.05$, nested ANOVA; Fig. 1). This was primarily driven by ants, of which density was significantly higher in the unlogged sites than in the RIL and CV sites ($p<0.05$, nested ANOVA; Fig. 2). The number of orders or equivalent taxonomic groups of macrofauna was similar across the sites of three management categories (Table 1). The density of litter transformers was significantly higher in the CV sites than in the RIL and unlogged sites ($p<0.05$, nested ANOVA; Fig. 2). The density of predators was significantly higher in the CV sites than in the RIL sites ($p<0.05$, nested ANOVA; Fig. 2). The result of CCA demonstrated that the water content of litter layer and the basal area percentage of Dipterocarpaceae explained well the compositional variation of the soil macrofauna community among the sites (Fig. 3). The water content of litter and soil has been suggested as an important limiting factor for the survival of some soil animal groups (e.g., Lavelle et al. 2001). On the other hand, the dominance of Dipterocarpaceae, which has been detected as an environmental variable closely related to the structure of soil macrofauna community in tropical forests, probably reflects the forest maturity. Along the process of forest maturation, changes in a number of associated environmental factors would govern the dynamics of soil macrofauna community.

Soil mesofauna

The density and the species richness of total Collembola and oribatid mites did not differ so distinctly among the ten sites (Table 2). As for the functional groups of Collembola, detritus feeders and fungal feeders did not show significant differences in density among the management categories (Fig. 4), but the density of sucking feeders was significantly higher in the unlogged sites than in the other sites ($p<0.05$, nested ANOVA; Fig. 4). Hasegawa (2006) suggested that sucking feeders decreased in clear-cut sites and that their species composition was well related to the changes in vegetation. Sucking feeders feed on bacteria and/or the organic matter in free soil water, and therefore may be sensitive to a drought on the forest floor, which is often caused by a decrease of vegetation cover in logged sites. These results suggest that the density and the species richness of the total mesofauna were not so much affected by logging within the magnitude of current harvest schemes applied in DFR. However, the composition of Collembolan feeding groups was affected by logging, regardless of RIL or CV.

In DFR, we did not find distinct effects of logging on the density and the number of taxonomic groups in soil macrofauna (excluding ants) and mesofauna. However, the composition of functional and/or taxonomic groups varied among the sites under different forest management schemes. Various degrees of logging disturbance caused by these managements must have differently affected the relative abundances of component soil animals through changes in the above-ground plant community and/or the water content of organic matter on forest floor. We suggest that the compositional structure of soil animal community can be a potential indicator for evaluating the soundness of various logging schemes in relation to ecosystem functioning and biodiversity conservation in tropical forests.

Acknowledgments

We express our thanks to the members of Forest Research Centre and Deramakot Forest Reserve for providing us with all the conveniences in the study.

References

Chey VK, Holloway JD, Hambler C, Speight MR (1998) Canopy knockdown of arthropods in exotic plantations and natural forests in Sabah, north-east Borneo, using insecticidal mist-blowing. Bull Ent Res 88:15-24

Chey VK, Holloway JD, Speight MR (1997) Diversity of moths in forest plantations and natural forests in Sabah. Bull Ent Res 87:371-385

Chung AYC, Eggleton P, Speight MR, Hammond PM, Chey VK (2000) The diversity of beetle assemblages in different habitat types in Sabah, Malaysia. Bull Ent Res 90:475-496

Eggleton P, Homathevi R, Jones DT, MacDonald JA, Jeeva D, Bignell DE, Davies RG, Maryati M (1999) Termite assemblages, forest disturbance and greenhouse gas fluxes in Sabah, East Malaysia. Phil Trans R Soc Lond B 354:1791-1802

Hasegawa M, Fukuyama K, Makino S, Okochi I, Goto H, Mizoguchi T, Sakata T, Tanaka H. (2006) Collembolan community dynamics during deciduous forests regeneration in Japan. Pedobiologia 50: 117-126

Lavelle P (2001) Soil Ecology. Kluwer Academic Publishers, Dordrecht

Lavelle P, Lattaud C, Trigo D, Barois I (1995) Mutualism and biodiversity in soils. Plant and soil 170:20-33

ter Braak CJF (1986) Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. Ecology 67:1167-1179

Willott SJ, Lim DC, Compton SG, and Sutton SL (2000) Effects of selective logging on the butterflies of a Bornean Rainforest. Conserv Biol 14:1055-1065

Table 1 Mean densities (m^{-2}) of soil macrofauna in ten sites under different forest management schemes.

Numbers in fig. 3	Soil macrofauna group	Forest management category									
		Unlogged				RIL				CV	
		PRI-1	PRI-2	80s-1	80s-2	RIL00-1	RIL00-2	RIL95-1	RIL95-2	CV-1	CV-2
	Platyhelminthes	0	0	0	0	0	0	0	0	3.2	0
32	Gastropoda	3.2	0	0	0	0	0	0	0	0	0
31	Hirudinea	0	0	0	3.2	0	0	3.2	0	0	0
	Oligochaeta (Earthworm)	83.2	12.8	48	9.6	28.8	22.4	32	48	64	38.4
	Pseudoscorpiones	28.8	35.2	22.4	28.8	12.8	3.2	38.4	25.6	35.2	35.2
28	Opiliones	3.2	6.4	3.2	0	0	3.2	0	6.4	3.2	3.2
	Araneae (Spider)	25.6	57.6	57.6	25.6	16	35.2	60.8	60.8	64	70.4
	Prostigmata	9.6	9.6	3.2	6.4	3.2	3.2	35.2	16	12.8	0
	Gamasida	3.2	12.8	0	3.2	0	3.2	16	9.6	9.6	9.6
	Oribatida	32	16	3.2	3.2	0	3.2	9.6	0	16	6.4
	Isopoda	28.8	12.8	9.6	19.2	6.4	12.8	16	9.6	28.8	73.6
	Diplopoda	32	3.2	19.2	19.2	6.4	12.8	9.6	12.8	25.6	57.6
27	Symphyla	3.2	6.4	6.4	0	0	3.2	0	0	3.2	6.4
	Lithobiomorpha	0	6.4	0	16	6.4	6.4	9.6	3.2	9.6	25.6
	Geophilomopha	6.4	6.4	9.6	6.4	3.2	3.2	9.6	3.2	6.4	6.4
	Collembola	48	67.2	115.2	28.8	32	44.8	112	92.8	57.6	73.6
	Campodeidae	6.4	9.6	16	0	0	3.2	3.2	9.6	22.4	22.4
	Japygidae	12.8	0	0	0	0	0	3.2	3.2	6.4	16
29	Thysanura	3.2	0	3.2	0	0	0	3.2	9.6	0	6.4
	Isoptera (Termite)	688	12.8	70.4	6.4	0	0	3.2	99.2	22.4	0
23	Blattodea	9.6	3.2	0	6.4	0	3.2	9.6	0	9.6	12.8
30	Dermaptera	0	0	0	0	0	0	0	0	6.4	0
25	Other Orthopetra	16	0	6.4	0	0	3.2	0	0	3.2	6.4
7	Hemiptera	22.4	48	41.6	6.4	19.2	9.6	28.8	32	19.2	22.4
22	Lepidoptera (larva)	12.8	6.4	19.2	3.2	0	3.2	3.2	0	0	6.4
15	Pselaphinae	19.2	19.2	0	0	3.2	0	22.4	3.2	28.8	0
	Staphylininae	28.8	9.6	28.8	3.2	9.6	28.8	6.4	19.2	3.2	3.2
10	Other Coleoptera (adult)	32	22.4	16	9.6	28.8	9.6	32	19.2	3.2	28.8
11	Other Coleoptera (larva)	22.4	6.4	19.2	12.8	9.6	12.8	25.6	57.6	22.4	9.6
19	Diptera (larva)	19.2	3.2	6.4	16	0	3.2	12.8	6.4	3.2	0
	Hymenoptera (Ants adult)	5357	828.8	656	176	131.2	211.2	172.8	67.2	73.6	364.8
	Hymenoptera (Ants larva)	25.6	0	0	3.2	9.6	9.6	0	3.2	0	166.4
	Insecta (unidentified)	6.4	9.6	0	6.4	0	0	0	0	0	6.4
	Total	6589	1232	1181	419.2	326.4	454.4	678.4	617.6	563.2	1078
	Total – ants	1206	403.2	524.8	240	185.6	233.6	505.6	547.2	489.6	547.2
	Number of groups	27	24	21	21	14	22	24	21	26	23

Table 2 Mean densities and species richness of Collembola in ten sites under the three categories of forest management schemes.

Management category	Site	Density (m^{-2})		Species richness		
		Average	SE	Average (per core)	SE	Total (per site)
unlogged	PRI-1	11000	1809	9.8	1.1	29
unlogged	PRI-2	9150	1282	9.8	0.9	33
unlogged	80s-1	12250	1988	10.9	1.4	34
unlogged	80s-2	16350	4104	11.4	1.1	34
RIL	RIL95-1	14000	2976	12.1	1.2	35
RIL	RIL95-2	7100	1668	7.9	1.2	33
RIL	RIL00-1	9900	2226	8.2	1.0	27
RIL	RIL00-2	10150	1886	9.3	0.9	28
CV	CV-1	7850	1883	7.9	1.1	28
CV	CV-2	16750	3366	11.1	1.1	35

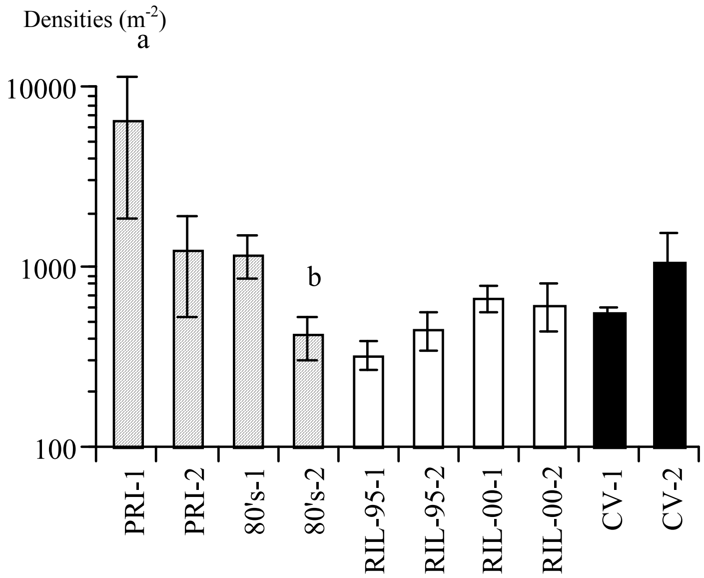


Fig. 1 Mean densities (bars: ±SE) of soil macrofauna in ten sites under the three categories of forest management schemes: “unlogged” (hatched), “RIL” (white), and “CV” (black).

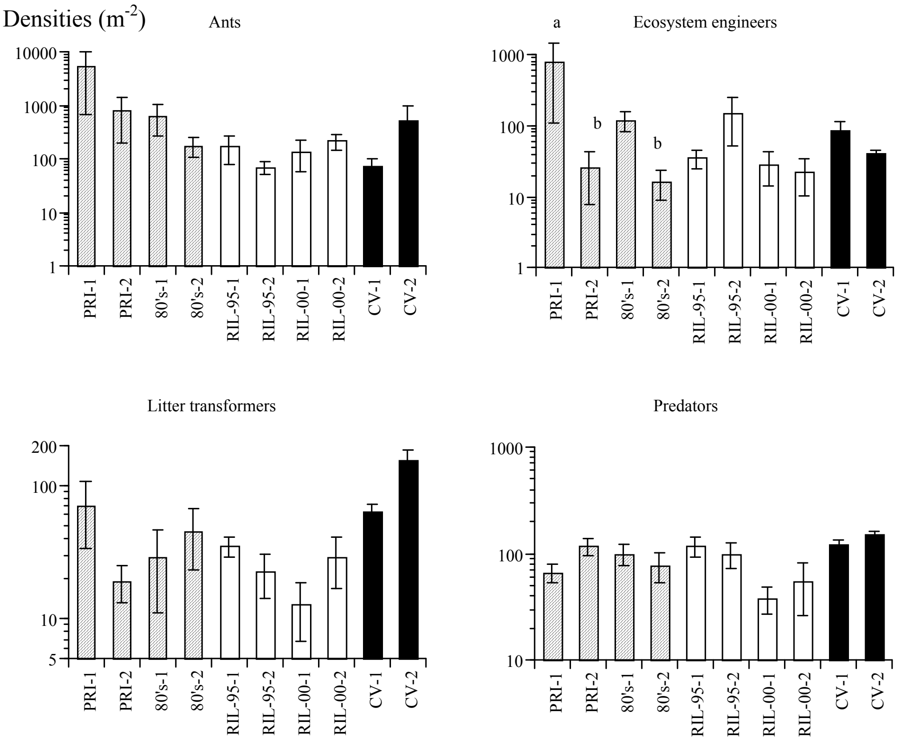


Fig. 2 Mean densities of four soil macrofauna functional groups (ants, ecosystem engineers, litter transformers and predators) in ten sites (see Fig. 1 for other explanations).

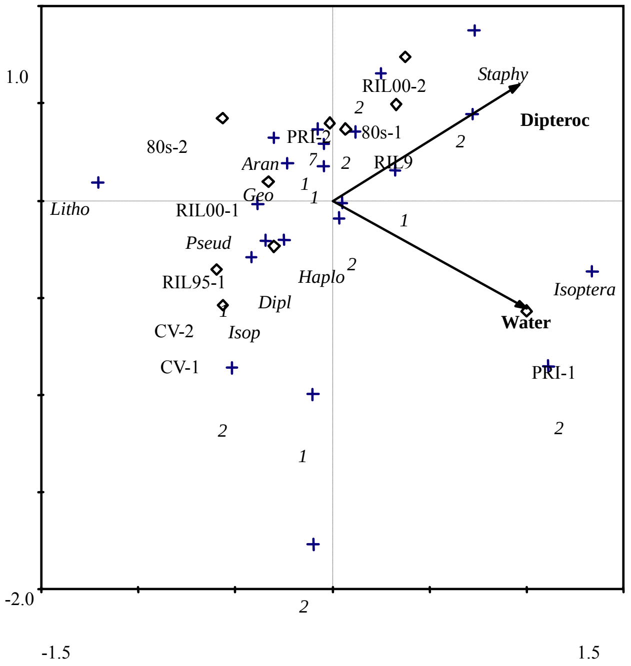


Fig. 3 Tri-plot ordination by CCA for soil macrofauna: sites (diamonds), taxonomic groups (crosses), and significant environmental variables (arrows). Dipteroc, relative basal area of Dipterocarpaceae; Water, water content in litter layer; Staphy, Staphylininae; Aran, Araneae; Litho, Lithobiomorpha; Pseud, Pseudscorpiones; Dipl, Diplopoda; Isopod, Isopoda; Haplo, Ologochaeta. Positions of other animal taxa are shown with numerals shown in Table 1.

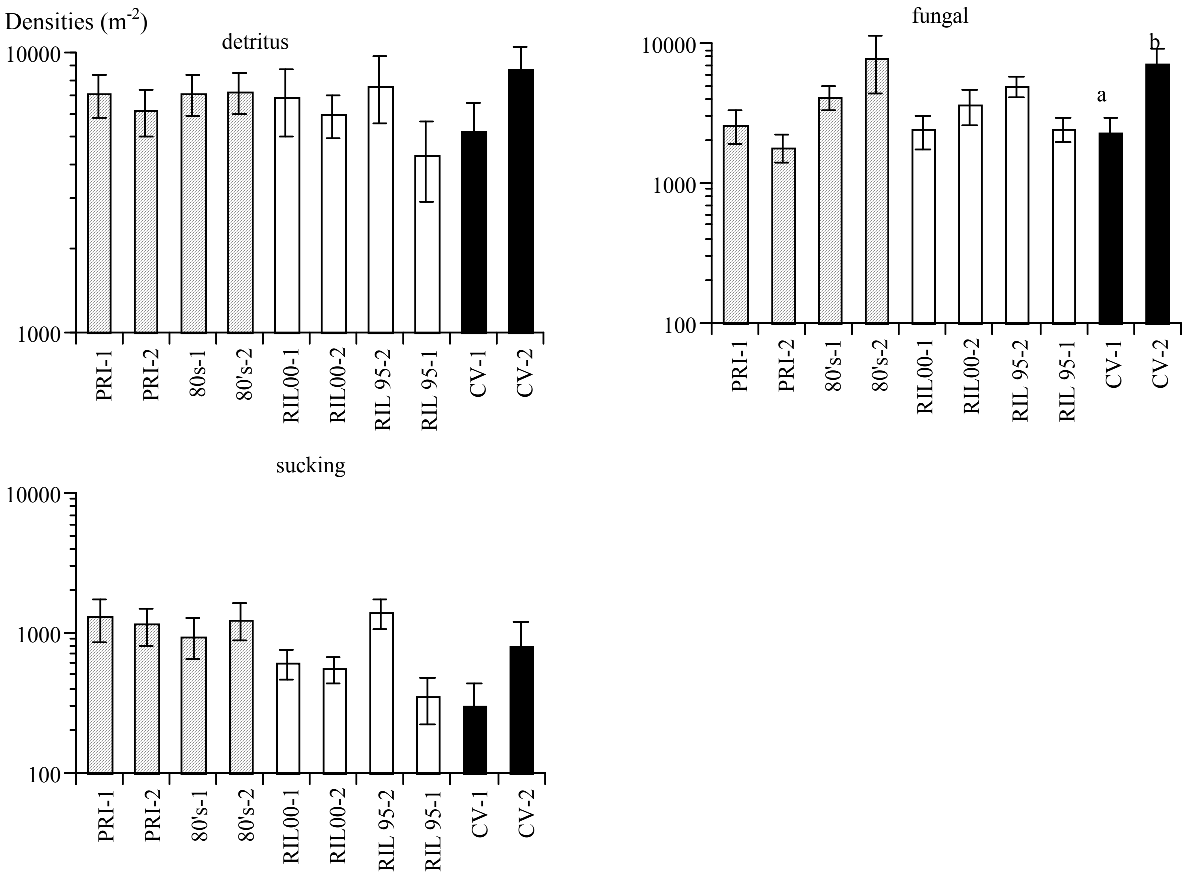


Fig. 4 Mean densities of three Collembolan feeding groups (detritus feeders, fungal feeders and sucking feeders) in ten sites (see Fig. 1 for other explanations).

Comparative Study on Mammalian Fauna in Different Harvesting Intensities with Reduced-Impact and Conventional Logging in Sabah, Malaysia

Go Onoguchi[1], Hisashi Matsubayashi[1, 2]
[1]Center for Ecological Research, Kyoto University
[2]Present, Tokyo University of Agriculture

Introduction

Habitat destruction including logging and forest clearing for agriculture has always been a major threat for most mammals in Southeast Asia and other parts of tropical regions (Cuaron 2000). Large proportion of tropical production forests (i.e. zoned for timber production) has already become logged-over forests, on which much of its biodiversity depends now (Frumfoff 1995). We must establish a strategy to conserve these threatened animals in production forests as well as those in primary forests (Frumhoff 1995; Johns 1997; Meijaard et al. 2006).

Selective logging is one of the most prevalent land uses in Southeast Asia and other tropical regions. The method is known to severely damage the residual stand and affect forest structure and biodiversity even though only a small proportion of the trees are often removed (Johns 1988; Cannon et al. 1994; Pinard and Putz 1996; Laurance and Laurance 1996).

There are several techniques for controlling and minimizing logging damage (e.g., Crome et al. 1992). One of such methods, reduced-impact logging (RIL), is a set of guidelines to reduce the physical impacts on the ground, remaining standing trees, streams and ecosystem as a whole with the combination of a pre-harvest census, carefully controlled felling and skidding, lowered allowable cut and regulated machinery use (Putz and Pinard 1993).

Some studies examined the effects of reduced-impact logging in retaining forest biomass and in damage reduction to forests in Southeast Asia and reported that reduced-impact logging reduced the damage or injury to residual stand by 18-27% (Pinard and Putz 1996; Bertault and Sist 1997; Sist et al. 1998). However, there is no study available on the mammalian responses to reduced-impact logging (Meijaard et al. 2006), although mammals have important ecological roles such as seed dispersal and/or pollination in the forests.

In this study, we examined the effects of reduced-impact logging on the mammalian fauna by comparing the diversity and the abundance in a reduced-impact logged forest and a conventionally logged forest in Sabah, Malaysia.

Materials and Methods

Study site

Our study was conducted in Deramakot Forest Reserve (55,083ha), a reduced-impact logged forest and adjacent Tangkulap Forest Reserve (27,550ha), a conventionally logged forest, in Sabah, Malaysian Borneo. The climate is humid equatorial with a mean annual temperature of about 26℃. Mean annual rainfall is about 3,500 mm (Huth and Ditzer 2001). The major vegetation of Deramakot is a mixed dipterocarp forest dominated by the family Dipterocarpaceae, while that of Tangkulap consists of abundant pioneer species of the genus *Macaranga* (Euphorbiaceae) (Seino et al. 2006).

Focal species and camera trap

Table 1 shows known middle- to large-sized mammal species in Deramakot (Matsubayashi et al. in press). We focused middle to large mammals because they are more likely to respond to landscape-level changes. Species of Chiroptera (bats), Dermoptera (colugo), small Insectivora (shrews), Scandentia (tree shrews), and small Rodentia (squirrels and rats) were excluded from this study. We follow the nomenclature by Payne et al. (1998).

A total of fourteen camera-traps were placed in the reduced-impact logged forest (7 traps) and the adjacent conventionally logged forest (7 traps) in February-March and August-September, 2006. In each forest, cameras were set up along animal trails at intervals of about 1km. We used automatic camera-traps (sensor camera Field note □, Marif, Yamaguchi, Japan) triggered by passive infrared motion sensors. All camera-traps were mounted on trees and set approximately 50cm from the ground, and were baited with durians or chicken, which were hung so high that animals could not consume them easily. All camera-traps operated 24 hours/day or until the film was fully exposed. We checked camera-trap sites every week to replace bait, and changed films and batteries if necessary. Day and time were recorded on each photograph.

We identified each photographed animal to species. As for genera *Muntiacus* (*Muntiacus atherodes* and *Muntiacus muntjak*) and *Tragulus* (*Tragulus napu* and *Tragulus javanicus*), we combined the congeneric species for each because they are indistinguishable on photographs. To exclude repetitive shots within a visit, we defined photographs of the same species within 60 minutes as 1 event. Number of camera-days was calculated for each camera trap. Since photographic rates correlate with animal density (Carbone et al. 2001), we used the number of independent photographs per camera-day as relative-abundance index (O'Brien 2003). In addition to camera-trap data, simultaneous field observations of larger mammals by night walking, driving census, and trace existence were also added to the species diversity list (Table 1).

We defined CNV/RIL ratio as the ratio between the relative-abundance index of each animal in the conventionally logged forest and that in the reduced-impact logged forest in order to discuss the relation between diet type and the persistence to heavy disturbance.

Differences in photographic rates between two forests were tested statistically using the Mann-Whitney *U* tests for each species recorded.

Results

Total study effort was 797 camera-days. Figure 1 shows species accumulation carves in each forest. Total 158 photographs were taken, of which 109 (396 camera-days) were from the reduced-impact logged forest and 49 (401 camera-days) were from the conventionally logged forest. In addition to the photographs of animals, there were 6 human records in conventionally logged forest.

Camera-trapped mammals consisted of 19 species, one Insectivora, three Primates, three Rodentia, eight Carnivora, and four Artiodactyla (Table 1). Of these 19 species, 18 species appeared in the reduced-impact logged forest and 11 in the conventionally logged forest (Table 1). Six species including sun bear and clouded leopard were recorded only in the reduced-impact logged site whereas only short-tailed mongoose was not detected in the reduced-impact logged forest.

The most numerous species trapped was mouse-deer *Tragulus* spp. (29 photos) followed by Malay civet *Viverra tangalunga* (27 photos), bearded pig *Sus barbatus* (24 photos), and pig-tailed macaque *Macaca nemestrina* (21 photos). Muntjac *Muntiacus* spp. and pig-tailed macaque *Macaca nemestrina* showed a significantly higher photographic rate in the reduced-impact logged forest ($p<0.05$; $p<0.05$), while no species were significantly more abundant in the conventionally logged forest. Total photographic rate was also significantly larger in the reduced-impact logged site ($p<0.05$). CNV/RIL was lower in frugivorous primates and higher in omnivorous pigs or carnivorous civets.

Discussion

Difference in mammalian fauna and abundance

The forest harvested by reduced-impact logging showed greater species richness than the forest logged conventionally (Table 1). However, the observed difference in the number of detected species may just reflect lower animal density in the conventionally logged forest but not the species number per se. Comparative studies of mammalian fauna in several paired sites of closely located logged and unlogged forests in Indonesia and Peninsular Malaysia showed that species presence was similar between logged and unlogged forests in some areas although slight differences were observed (Johns 1997; Laidlaw 2000; Wilson and Johns 1982).

The results suggest that forests exploited using reduced-impact logging is able to carry a higher density of the middle to large mammals compared with forests logged conventionally. A previous study also revealed that the density of Bornean orangutan *Pongo pygmaeus* in our focal reduced-impact logged site (Deramakot) was 1.50 individuals/km^2, which was more than twice as high as 0.62 individuals/km^2 in the site logged conventionally (Tangkulap) (Ancrenaz et al. 2005). Earlier studies have already documented that large mammals in Borneo often become less abundant in selectively logged forests (Felton et al. 2003; Heydon and Bulloh 1996, 1997).

Effect of difference in habitat quality and human presence

Table 2 shows the summary of results from current and another study in Deramakot and diet type for each species. Among the six species listed here, two primates are the most fruit-dependent animals, mouse-deer and muntjac are less dependent, and bearded pig and Malay civet are the least. CNV/RIL was lower in frugivorous primates and higher in omnivorous pigs or carnivorous civets. This tendency that frugivorous animals are more vulnerable to logging and omnivores or carnivores are tolerant indicates that heavy logging reduces fruit production in forest and reduces animal population consequently. It is known that primates' degree of frugivory negatively correlates with species' persistence to logging (Johns and Skorupa 1987). Logging activities reduce the availability of food resources for frugivores, even where timber trees are not themselves used by animals (Johns 1988; but see Ganzhorn 1995). Poor logging operation in the conventional method may cause the reduction in food resources and negatively affect the abundance of mammals.

In addition to food habitat, Marsh et al. (1987) suggested that the degree of territoriality could influence the adaptability of animals. Some primates and civets in Deramakot were strictly arboreal, although they were hardly recorded in this survey. Increased amount of canopy gap disrupts aerial pathway and arboreal species experience difficulties in locomotion (Johns 1997). Therefore, arboreal mammals can be more susceptible to logging.

Human activities accompanying logging practices can also affect mammals. It is known that hunting poses a great threat to large forest animals in many parts of the tropics (Linkie et al. 2003; Marshall et al. 2006). It can be even a greater threat to wildlife than timber harvesting in some cases (Bennet et al. 2002; Matthews and Matthews 2002; Walsh et al. 2003). In Deramakot and adjacent Tangkulap, hunting of wildlife is prohibited, but illegal hunting still occurs by villager and outsiders. Hunting pressure was higher in Tangkulap (conventionally logged area) because of easier access and insufficient prevention there. Therefore, Sabah Forestry Department has enforced regulation on the illegal hunting in Tangkulap area since 2005 (P. Lagan, Assistant District Forestry Officer, Deramakot, Sabah Forestry Department, pers. comm.).

Reduced-impact logging and the regulation of illegal hunting could maintain the food resources for mammals and secure their population. Further investigation is needed on the logging impact on the ecological functions of mammals in addition to that on the population density.

References

Ancrenaz M, Gimenez O, Ambu L, Ancrenaz K, Andau P, Goossens B, Payne J, Sawang A, Tuuga A, Lackman-Ancrenaz I (2005) Aerial surveys give new estimates for orangutans in Sabah, Malaysia. PLoS Biology, 3, 1-8.

Bennett EL, Milner-Gulland EJ, Bakarr M, Eves HE, Robinson JG, and Wilkie D S (2002) Hunting the world's wildlife to extinction. Oryx, 36, 328-329.

Bertault J, Sist P (1997) An experimental comparison of different harvesting intensities with reduced-impact and conventional logging in East Kalimantan, Indonesia. Forest Ecology and Management, 94, 209-218.

Caldecott JO (1986) An ecological and behavioral study of Pig-tailed Macaques. Karger, Basel, Switzerland.

Cannon CH, Peart DR, Leighton M, Kartawinata K (1994) The structure of lowland rainforest after selective logging in West Kalimantan, Indonesia. Forest Ecology and Management, 67, 49-68.

Carbone C, Christie S, Conforti K, Coulson T, Franklin N, Ginsberg JR, Griffiths M, Holden J, Kawanishi K, Kinnaird M, Laidlaw R, Lynam A, Macdonald DW, Martyr D, McDougal C, Nath L, O'Brien T, Seidensticker J, Smith DJL, Sunquist M, Tilson R, Wan Shahruddin WN (2001) The use of photographic rates to estimate densities of tigers and other cryptic mammals. Animal Conservation, 4, 75-79.

Crome FHJ, Moore LA, Richards GC (1992) A study of logging damage in upland rainforest in north Queensland. Forest Ecology and Management, 49, 1-29.

Cuaron AD (2000) A global perspective on habitat disturbance and tropical rainforest mammals. Conservation Biology 14: 1574-1579.

Davis DD (1962) Mammals of the lowland rain-forest of North Borneo. Bulletin of the Singapore Natural History Museum, 31, 1-129.

Felton AM, Engstrom LM, Felton A & Knott CD (2003) Orangutan population density, forest structure and fruit availability in a hand-logged and unlogged peat swamp forests in West Kalimantan, Indonesia. Biological Conservation, 114, 91-101.

Frumuhoff PC (1995) Conserving wildlife in tropical forests managed for timber to provide a more viable complement to protected areas. BioScience 45: 456-464.

Ganzhorn JU (1995) Low-level forest disturbance effects on primary production, leaf chemistry, and lemur populations. Ecology, 76, 2084-2096.

Heydon MJ, Bulloh P (1996) The impact of selective logging on sympatric civet species in Borneo. Oryx, 30, 31-36.

Heydon MJ, Bulloh P (1997) Mousedeer densities in a tropical rainforest: the impact of selective logging. Journal of Applied Ecology, 34, 484-496.

Huth A, Ditzer T (2001) Long-term impacts of logging in a tropical rain forest – a simulation study. Forest Ecology and Management, 142, 33-51.

IUCN (2006) IUCN Red List of Threatened Species. www.iucnredlict.org

Johns AD, Skorupa JP (1987) Responses of rain-forest primates to habitat disturbance: a review. International Journal of Primatology, 8, 157-191.

Johns AD (1988) Effect of "selective" timber extraction on rain forest structure and composition and some consequences for frugivores and folivores. Biotropica, 20, 31-37.

Johns AG (1997) Timber production and biodiversity conservation tropical rain forests. Cambridge University Press, UK.

Kinnarird MF, Sanderson EW, O'Brien TG, Wibisono HT, and Woolmer G (2003) Deforestation trends in a tropical landscape and implications for endangered large mammals. Conservation Biology 17: 245-257.

Laidlaw RK (2000) Effects of habitat disturbance and protected areas on mammals of Peninsular Malaysia. Conservation Biology, 14, 1639-1648.

Laurance WF, Laurance SGW (1996) Responses of five arboreal marsupials to recent selective logging in tropical Australia. Biotropica, 28, 310-322.

Linkie M, Martyr DJ, Holden J, Yanuar A, Hartana AT, Sugardjito J, Leader-Williams N (2003) Habitat destruction and poaching threaten the Sumatran tiger in Kerinci Seblat National Park, Sumatra. Oryx, 37, 41-48.

Marsh CW, Johns AD, Ayres JM (1987) Effects of habitat disturbance on rain forest primates. In: Marsh CW, Mittermeier RA editors. Primate conservation in the tropical rain forest. Alan R. Liss, New York. Pp. 83-107.

Marshall AJ, Nardiyono, Engström LM, Pamungkas B, Palapa J, Meijaard E & Stanley SA (2006) The blowgun is mightier than the chainsaw in determining population density of Bornean orangutans (*Pongo pygmaeus morio*) in the forests of East Kalimantan. Biological Conservation, 129, 566-578.

Matsubayashi H, Lagan P, Majalap N, Tangah J, Sukor JRA, Kitayama K (in press) Importance of natural licks for the mammals in Bornean inland tropical rain forests. Ecological Research. DOI: 10.1007/s11284-006-0313-4

Matthews A, Matthews A (2002) Distribution, population density, and status of sympatric Cercopithecids in the Campo-Ma'an area, Southwestern Cameroon. Primates, 43, 155-168.

Meijaard E, Sheil D, Nasi R, Stanley SA (2006) Wildlife conservation in Bornean timber concessions. Ecology and Society, 11(1), 47

O'Brien TG, Kinnaird MF, Wibisono HT (2003) Crouching tigers, hidden prey: Sumatran tiger and prey populations in a tropical forest landscape. Animal Conservation, 6, 131-139.

Payne J, Francis CM, Phillipps K (1985) A field guide to the mammals of Borneo. The Sabah society with world wildlife fund Malaysia, Kota Kinabalu

Pinard MA, Putz FE (1996) Retaining forest biomass by reducing logging damage. Biotropica, 28, 278-295.

Putz FE, Pinard MA (1993) Reduced-impact logging as a carbon offset method. Conservation Biology, 7, 755-757.

Seino T, Takyu M, Aiba S, Kitayama K, Ong R (2005) Floristic composition, stand structure, and above-ground biomass of the tropical rain forests of Deramakot and Tangkulap Forest Reserve in Malaysia under different forest managements. In: Lee YF, Chung AY & Kitayama K editors. Proceedings of The 2nd Workshop on Synergy between Carbon Management and Biodiversity Conservation in Tropical Rain Forests. DIWPA, Kyoto. Pp. 29-52.

Walsh PD, Abernethy KA, Bermejo M, Beyersk R, Wachter PD, Akou ME, Huijbregts B, Mambounga D, Toham AK, Kilbournk AM, Lahmq SA, Latourk S, Maiselsk F, Mbinak C, Mihindouk Y, Obiang SN, Effa EN, Starkeyk MP, Telfer P, Thibault M, Tutin CEG, Whitek LJT, Wilkiek DS (2003) Catastrophic ape decline in western equatorial Africa. Nature, 422, 611-614.

Wilson WL, Johns AD (1982) Diversity and abundance of selected animal species in undisturbed forest, selectively logged forest and plantations, in East Kalimantan, Indonesia. Biological Conservation, 24, 205-218.

Table 1. Known middle – large mammals in Deramakot and summary of photographic records from the reduced-impact logged forest (RIL) and the adjacent conventionally logged forest (CNV).

		Records/camera-day		
Species [a]	Common names	RIL	CNV	IUCN 2006
INSECTIVORA				
Erinaceidae				
Echinosorex gymnurus	Moonrat	0.005	N/A	LC
PRIMATES				
Lorisidae				
Nycticebus coucang	Slow loris	N/A	N/A	LC
Tarsiidae				
Tarsius bancanus	Western tarsier	0.002	0.002	LC
Cercopithecidae				
Presbytis rubicunda	Red leaf monkey	N/A	N/A	LC
Presbytis cristata	Silvered langur	N/A	N/A	Not listed
Nasalis larvatus	Proboscis monkey	N/A	N/A	EN A2c, C1+2a
Macaca fascicularis	Long-tailed macaque	0.002	0.002	NT
Macaca nemestrina	Pig-tailed macaque	0.043 *	0.010	VU A1cd
Hylobatidae				
Hylobates muelleri	Bornean gibbon	N/A	N/A	NT
Pongidae				
Pongo pygmaeus	Orangutan	N/A	N/A	EN A2cd
PHOLIDOTA				
Manidae				
Manis javanica	Pangolin	N/A	N/A	NT
RODENTIA				
Hystricidae				
Trichys fasciculate	Long-tailed porcupine	0.005	N/A	LC
Hystrix brachyuran	Common porcupine	0.012	N/A	VU A1d
Thecurus crassispinis	Thick-spined porcupine	0.008	N/A	NT
CARNIVORA				
Ursidae				
Helarctos malayanus	Sun bear	0.010	N/A	DD
Mustelidae				
Martes flavigula	Yellow-throated marten	N/A	N/A	LC
Mydaus javanensis	Malay badger	0.005	0.002	LC
Aonyx cinerea	Oriental small-clawed otter	N/A	N/A	NT
Viverridae				
Viverra tangalunga	Malay civet	0.040	0.026	LC
Cynogale bennettii	Otter-civet	N/A	N/A	EN A1ce, C2a
Arctictis binturong	Binturong	0.008	0.005	LC
Arctogalidia trivirgata	Small-toothed palm civet	N/A	N/A	LC
Paguma larvata	Masked Palm civet	N/A	N/A	LC
Paradoxurus hermaphroditus	Common palm civet	0.010	0.007	LC
Hemigalus derbyanus	Banded palm civet	0.003	N/A	LC
Herpestidae				
Herpestes brachyurus	Short-tailed mongoose	N/A	0.002	LC
Herpestes semitorquatus	Collared mongoose	N/A	N/A	LC
Felidae				
Neofelis nebulosa	Clouded leopard	0.002	N/A	VU C2a(i)
Prionailurus planiceps	Flat-headed cat	N/A	N/A	VU C2a(i)
Prionailurus bengalensis	Leopard cat	N/A	N/A	LC

[a] Listed by Matsubayashi et al. (in press). *Arctogalidia trivirgata* was added. [b] Two species were pooled. * Significantly more abundant, $P < 0.05$IUCN status of each species is also given: EN-endangered; VU-vulnerable; NT-near threatened; LC-least concern; DD-data deficient

Table 1. Known middle – large mammals in Deramakot and summary of photographic records from the reduced-impact logged forest (RIL) and the adjacent conventionally logged forest (CNV).(*Continued*)

Species [a]	Common names	Records/camera-day RIL	Records/camera-day CNV	IUCN 2006
PROBOSCIDEA				
Elephantidae				
Elephas maximus	Asian elephant	N/A	N/A	EN A1cd
ARTIODACTYLA				
Suidae				
Sus barbatus	Bearded pig	0.040	0.019	LC
Tragulidae		0.050 [b]	0.025 [b]	
Tragulus javanicus	Lesser mouse-deer			LC
Tragulus napu	Greater mouse-deer			LC
Cervidae		0.025 [*] [b]	N/A	
Muntiacus atherodes	Bornean yellow muntjac			LC
Muntiacus muntjak	Red muntjac			LC
Cervus unicolor	Sambar deer	0.005	0.003	LC
Bovidae				
Bos javanicus	Tembadau / Banteng	N/A	N/A	EN A1cd+2cd, C1+2a
	TOTAL	0.277 [*]	0.105	

[a] Listed by Matsubayashi et al. (in press). *Arctogalidia trivirgata* was added. [b] Two species were pooled. [*] Significantly more abundant, $P < 0.05$IUCN status of each species is also given: EN-endangered; VU-vulnerable; NT-near threatened; LC-least concern; DD-data deficient

Table 2. Summary of mammalian density (as indexed by the relative-abundance index) in Deramakot and diet type

Survey method	Species	Density RIL	Density CNV	CNV / RIL	Diet type	% Frugivory (Source)
Camera-trapping [a]		records/camera-day				
	Mouse-deer	0.050	0.025	0.50	Frugivore/ Browser	
	Malay civet	0.040	0.026	0.65	Carnivore/ Insectivore	15% (Davis 1962)
	Bearded pig	0.040	0.019	0.48	Omnivore	
	Pig-tailed macaque	0.043	0.010	0.23	Frugivore	88% (Caldecott 1986)
	Muntjac	0.025	0.000	0	Frugivore/ Browser	
Aerial nest count [b]		individuals/km^2				
	Orangutan	1.50	0.62	0.41	Frugivore	100 - 21% (Knott 1998)

[a] Only species trapped > 9 times were listed.

[b] Data from Ancrenaz et al. (2005)

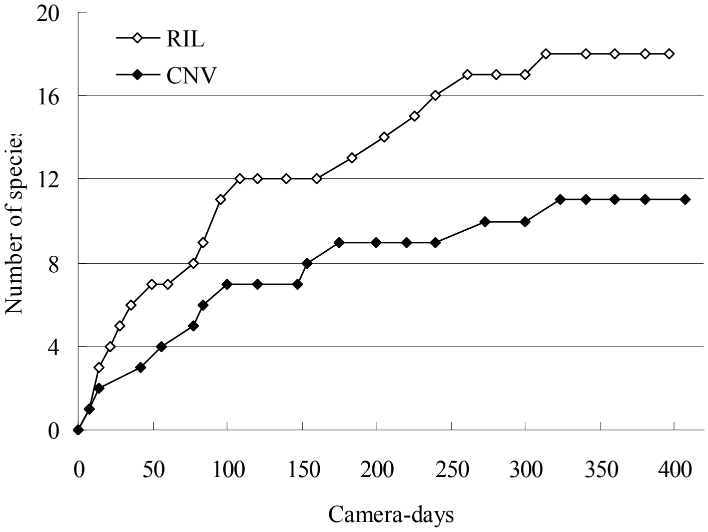


Fig. 1. Species accumulation carves in the reduced-impact logged forest (RIL) and the conventionally logged forest (CNV).

Tree Species Composition and Diversity in Relation to Forest Management in the Lowlands of Yakushima

Shin-ichiro Aiba[1], Cecilia Simoes[2], Naohiko Noma[3],
Masayuki Takamiya[4], Takashi Kohyama[5]
[1]Faculty of Science, Kagoshima University, Kagoshima 890-0065, Japan.
[2]Graduate School of Science and Engineering, Kagoshima University, Kagoshima, Japan.
[3]School of Environmental Science, University of Shiga Prefecture, Hikone, Shiga, Japan
[4]Graduate School of Science and Technology, Kumamoto University, Kumamoto, Japan
[5]Faculty of Environmental Earth Science, Hokkaido University, Sapporo, Japan

Introduction

We examined the effects of forest management on tree species composition and diversity in the lowland forests of Yakushima, an island in the south of Kyushu, Japan. Virtually all evergreen broad-leaved forests have been logged in Japan, but old-growth forests, which serve as a control treatment, still remain on Yakushima Island. Aiba et al. (2001) compared old-growth forests and secondary forests that were regenerating after the clear-cutting of old-growth forests on Yakushima Island over a range of altitude from 300 to 800 m above sea level. We performed a similar comparison, but restricted the altitude to lowland < 500 m to reduce the variation in environmental conditions, and included both conifer plantations and secondary forests that were regenerating after the clear-cutting of plantations. Conifer plantations have been converted from old-growth forests over the past 75 years or so and are currently one of the most dominant types of land use in the lowlands of Yakushima Island. In future, it may be desirable to reestablish natural forests after harvesting the plantations. However, little is known about what happens following the abandonment of plantations after harvesting (Sakai et al. 2006, Yamagawa and Ito 2006).

Materials and Methods

The study sites were located in the lowland forests (< 500 m above sea level) on Yakushima Island, Kagoshima Prefecture, Japan (30° N 130° E). The lowlands of Yakushima Island were once covered by old-growth evergreen broad-leaved forests. Most of the old-growth forests had been clear-cut by the early 1930s, and plantations of the evergreen conifer *Cryptomeria japonica* were established mainly on lower slopes (Fig. 1). Secondary forests also regenerated in clear-cuts that were not planted with *C. japonica*. Recently, these *Cryptomeria* plantations were again logged, but further afforestation was not conducted. In such areas, low-diversity forests strongly dominated by the deciduous broad-leaved tree *Aleurites cordata* regenerated. In addition to these silvicultural practices, topography affects the tree species composition in the lowland forests on Yakushima Island (Bellingham et al. 1996; Aiba et al. 2001, Tsujino et al. 2006), although *Cryptomeria* plantations and *Aleurites* forests are mainly restricted to lower slopes. We therefore established a total of 24 plots, with four in each of the following categories of forest: (1) old-growth forest on upper slopes, including ridges and steep mid-slopes; (2) old-growth forest on lower slopes, including valleys and gentle mid-slopes; (3) secondary forest on upper slopes; (4) secondary forest on lower slopes; (5) *Cryptomeria* plantation on lower slopes; and (6) *Aleurites* forest on lower slopes. The plot locations were selected in National Forests and privately owned forests using maps from the local Forestry Office and by interviewing local people, respectively.

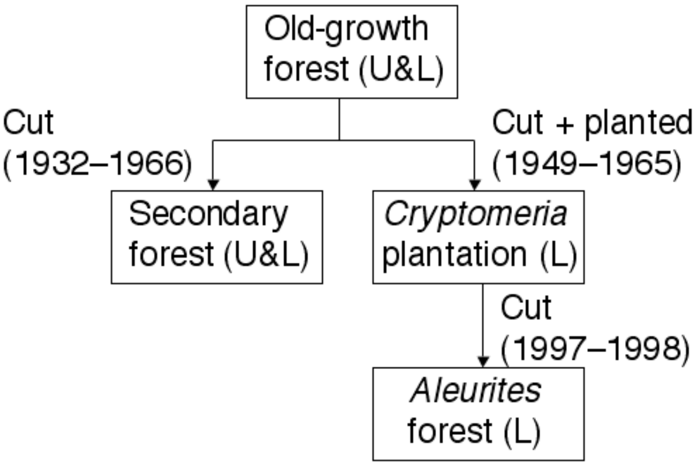


Fig. 1 The relationship among four forest types in relation to forest management in the lowlands of Yakushima Island. Year of logging for the study sites and the topography of each forest type (U, upper slope; L, lower slope) are also shown.

A tree census was conducted from 2006 to 2007. The plot area was 400 m^2 (10 × 40 m or 20 × 20 m), measured on the ground, depending on the availability of similar topography and the spatial extent of the forest type. All stems, including multiple stems, ≥ 2 cm in diameter at breast height (dbh, 1.3 m above the ground) were measured for dbh and identified to species. One old-growth plot on an upper slope, four secondary plots on upper slopes and two secondary plots on lower slopes had been established in 1984 (Kohyama 1988, 1989; Kohyama and Aiba 1997). Of these, two secondary plots on upper slopes and two secondary plots on lower slopes had an initial area of 100 m^2; these plots were expanded to 400 m^2 prior to the 2006–2007 census.

We conducted detrended correspondence analysis (DCA) to summarize the species composition (ter Braak and Smilauer 2002). We added 1 to the relative basal area of each species and log-transformed the input data. *Aleurites* forests (8–9 years old) were much younger than secondary forests (40–74 years old) in 2006–2007, but the secondary forests were 18–52 years old in 1984. We incorporated the 1984 data as well as the 2006–2007 data from the seven plots in the DCA to make the comparison between *Aleurites* forests and secondary forests more meaningful and to examine the temporal change in species composition over 22 years (all seven plots were recensused in 2006). We counted the number of species in each plot and computed Fisher's α, Shannon's index of diversity, and Pielou's index of evenness to evaluate species diversity; natural logarithms and stem numbers were used for the latter two indices (Magurran 2004). Fisher's α represents diversity in terms of the number of species relative to the number of stems, whereas Shannon's index is dependent on both the number of species and the evenness of species abundance.

Results and Discussion

Secondary stands showed forest structure similar to that of old-growth stands, except that the maximum dbh was significantly smaller on both upper and lower slopes and stem density was greater on upper slopes (Fig. 2), suggesting that forest structure had almost recovered to old-growth status 40–74 years after clear-cutting.

This is consistent with the results of Kohyama (1989) and Aiba et al. (2001). *Cryptomeria* plantations had a greater mean dbh than did the other forest types and tended to contain fewer stems per area. They also had a significantly greater basal area than did secondary forests of similar age on lower slopes (both approximately 40–50 years old). All of these differences likely reflect the effects of thinning management. *Aleurites* stands regenerating 8–9 years after the logging of *Cryptomeria* plantations had a significantly lower basal area (mean: 18.9 cm^2 m^{-2}) and maximum dbh (mean: 14.5 cm) than did other forest types. The basal area and maximum dbh of the six secondary forests for which long-term data were available were 39.9–53.9 cm^2 m^{-2} in 1984 (stand age 18–52 years old) and 15.3–39.1 cm, respectively. Therefore, the lower basal area of *Aleurites* forests may be characteristic of this forest type, but the smaller maximum dbh is probably a result of the younger age.

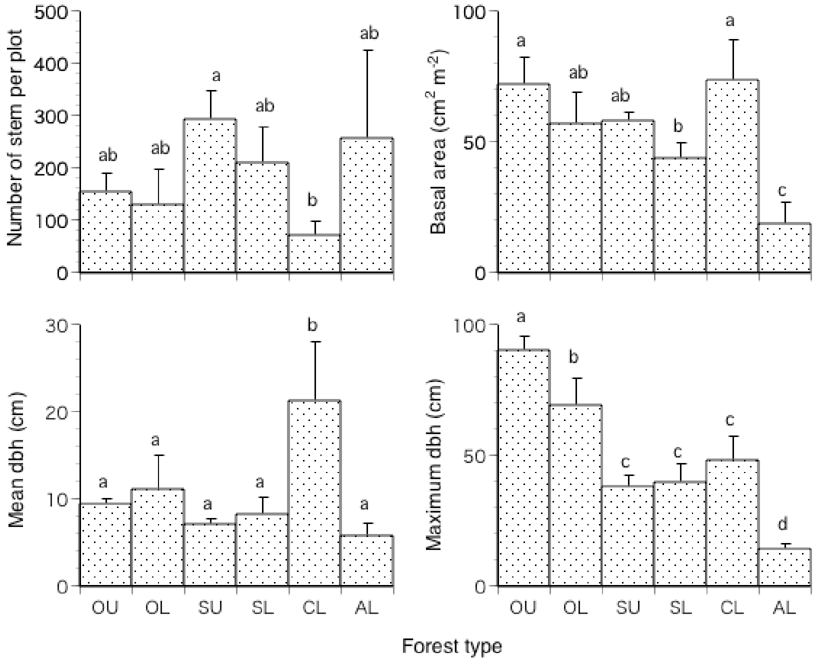


Fig. 2 Forest structure of the six forest-type categories in the lowlands of Yakushima Island.
The sample size was four plots in each forest-type category. Standard deviations are shown as vertical bars. Different letters indicate significant differences among plots at $P < 0.05$ using Tukey multiple comparisons (OU, old-growth forest on upper slope; OL, old-growth forest on lower slope; SU, secondary forest on upper slope; SL, secondary forest on lower slope; CL, *Cryptomeria* plantation on lower slope; AL, *Aleurites* forest regenerating on clear-cut *Cryptomeria* plantations on lower slope).

On upper slopes, both the old-growth and secondary plots had a high relative basal area (RBA >10%) of *Castanopsis cuspidata* var. *sieboldii*, which was the top dominant species in terms of basal area in three old-growth plots (RBA: 36–45%) and three secondary plots (36–69%). *C. cuspidata* probably regenerated from cut stamps in secondary forests, as suggested by the frequent occurrence of multiple-stemmed individuals. Other top dominants were *Quercus salicina* (RBA: 33%) in an old-growth plot and *Lithocarpus (Pasania) edulis* (36%) in a secondary plot. On lower slopes, no species showed consistently high RBA. The top dominants were *Q. salicina* (two plots, RBA: 23 and 51%), *Distylium racemosum* (27%), and *Schefflera octophylla* (23%) in old-growth plots, and *Aleurites cordata* (43%), *Ardisia sieboldii* (18%), *Fagara ailanthoides* (36%), and *Litsea acuminata* (31%) in secondary plots. As might be expected, plantations were strongly dominated by *Cryptomeria japonica*, exhibiting high RBA of 86–100%. One of the *Cryptomeria* plantations was well managed and contained only *C. japonica*. The other three plantations were less managed and contained other species, mainly in the understory. *Aleurites cordata* stands that developed after the logging of *Cryptomeria* plantations were strongly dominated by *A. cordata*, exhibiting high RBA of 42−100%.

We are aware of only two studies that have documented the natural vegetation regeneration after the clear-cutting of conifer plantations in the warm-temperate region of Japan. Yamagawa and Ito (2006) compared tree species composition before and after clear-cutting of *Chamaecyparis obtusa* plantations in Miyazaki Prefecture, Kyushu, and pointed out the importance of both advanced regeneration (surviving and resprouting stems) and new regeneration from seedlings; advanced regeneration reflected the abundant understory vegetation in the plantations. Sakai et al. (2006) found that evergreen oaks, including *C. cuspidata* and *Q. salicina*, successfully regenerated after clear-cutting of *C. japonica* and *C. obtusa* plantations in southern Shikoku and considered that this was caused by advanced regeneration. However, in the *Aleurites* forests of Yakushima, regeneration was very poor, probably because of poorly developed understory vegetation in the original plantation, where advanced regeneration appeared to be lacking. Only *Ardisia sieboldii*, which occurred as multi-stemmed individuals, had a potential for advanced regeneration by resprouting.

The eigenvalues of axes 1 and 2 of the DCA were 0.70 and 0.31, respectively, and cumulatively explained 27% of the variance. Along axis 1, three broad groups of forest were separated in order of increasing score (Fig. 3): (1) *Cryptomeria* plantations and *Aleurites* forests, (2) old-growth and secondary forests on lower slopes, and (3) old-growth and secondary forests on upper slopes. Along axis 2, further compositional differences were found between *Cryptomeria* plantations and *Aleurites* forests and between old-growth and secondary forests on lower slopes, but the difference between old-growth and secondary forests was unclear on upper slopes. Temporal changes in the species composition over 22 years were slight compared to the variation among the forest types *Aleurites* forests 8–9 years old were distinctively different from secondary forests both in 1984 (18–52 years old) and in 2006–2007 (40–74 years old).

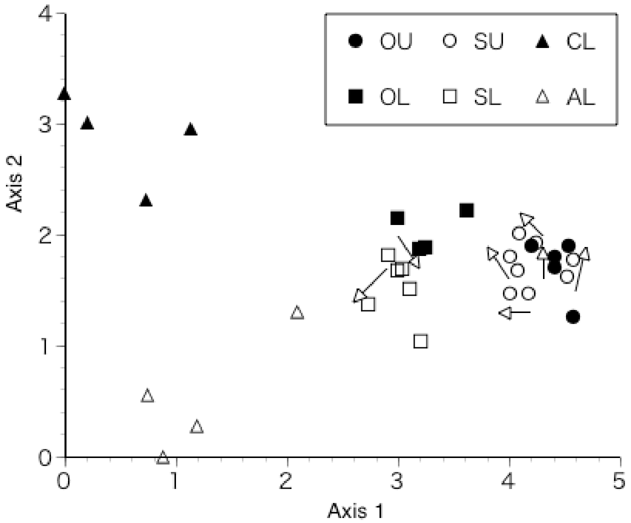


Fig. 3 DCA ordination diagram of plot scores. Arrows indicate the change in species composition from 1984 to 2006–2007 for seven long-term plots. Other plots were censused only in 2006–2007. See Fig. 2 for abbreviations of forest types.

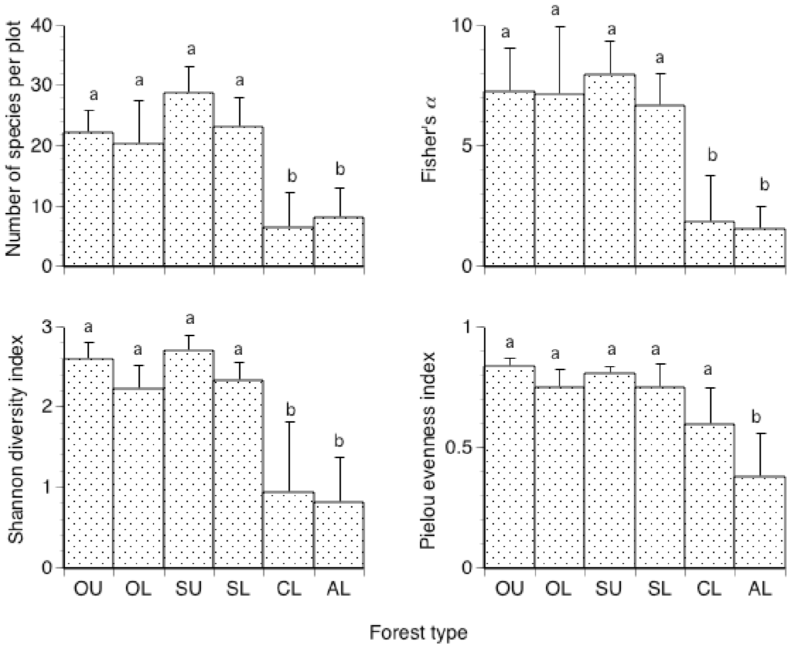


Fig. 4 Species diversity evaluated using various diversity indices for six forest-type categories. The sample size was four plots in each forest-type category, except for Pielou's index for *Cryptomeria* plantations for which n = 3 because one plantation plot contained only one species. Standard deviations are shown as vertical bars. Different letters indicate significant differences among plots at P < 0.05 using Tukey multiple comparisons. See Fig. 2 for abbreviations of forest types.

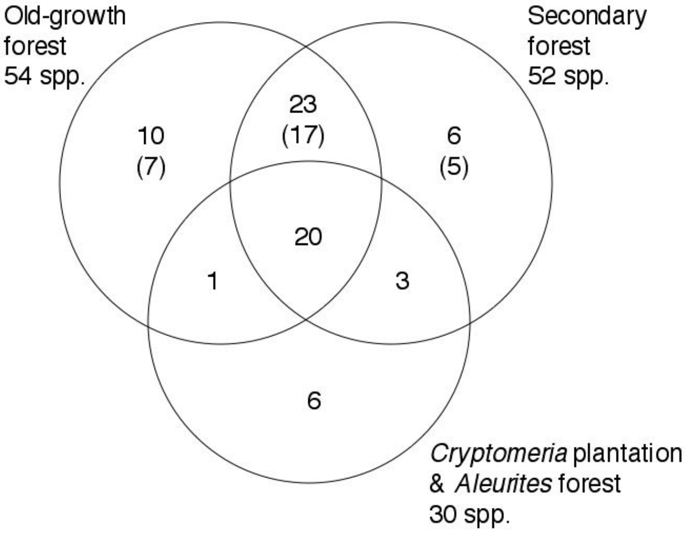


Fig. 5 Venn diagram of species occurrence in three forest-type groups, including old-growth forests on upper and lower slopes, secondary forests on upper and lower slopes, and *Cryptomeria* plantations and *Aleurites* forests (both on lower slopes). For species that are absent from the *Cryptomeria* and *Aleurites* forest group, the numbers of species that occurred on lower slopes are shown in parentheses.

Cryptomeria and *Aleurites* forests were less diverse than secondary and old-growth stands, although *Cryptomeria* plantations did not have significantly lower Pielou's index values than did old-growth or secondary forests (Fig. 4). One of the *Cryptomeria* plantations contained only *C. japonica*, and Pielou's index could not be calculated for this plantation. The differences between secondary and old-growth forests were unclear, which was consistent previous results (Aiba et al., 2001); the differences between upper and lower slopes were also unclear.

The 4484 stems ≥ 2 cm in dbh in the 24 plots (0.96 ha) censused in 2006–2007 comprised 69 species. We examined the distribution of species among three groups of forests: old-growth, secondary, and *Cryptomeria* plus *Aleurites*. As many as 39 (57%) of the 69 species were absent from the *Cryptomeria* plus *Aleurites* forest group (Fig. 5). Of these, 29 species occurred on lower slopes in old-growth or secondary forests or both, so that their absence from *Cryptomeria* and *Aleurites* forests was not entirely a result of habitat associations. Species that appeared to be unable to invade *Cryptomeria* or *Aleurites* forests included all three Fagaceae species (*Castanopsis cuspidata*, *Lithocarpus edulis*, and *Quercus salicina*) that bear large acorns and are dominant (RBA > 30%) in one or more plots of the old-growth and secondary forests. This indicates that dispersal limitation is partly responsible for the poor species diversity of *Cryptomeria* and *Aleurites* forests.

However, the *Aleurites* forests investigated were all located nearby (approximately 10–50 m) old-growth or secondary forests that can act as seed sources. Thus, dispersal limitation was not the primary reason for the poor species diversity of *Aleurites* forests. Poor advanced regeneration caused by intensive forest management such as thinning and weeding in the understory seemed to be an important factor. Post-harvest disturbance such as soil erosion and herbivory by sika deer may also be important (Sakai et al. 2006). Seeds of *A. cordata* are poisonous to humans, and the species' vegetative parts may also be toxic to mammals. Indeed, Tsujino and Yumoto (2004) did not find evidence of deer herbivory on *A. cordata* saplings in an old-growth lowland forest on Yakushima Island. A lack of herbivory by deer could explain the monodominance of *A. cordata* on clear-cuts of plantations.

References

Aiba S, Hill DA, Agetsuma N (2001) Comparison between old-growth stands and secondary stands regenerating after clear-felling in warm-temperate forests of Yakushima, southern Japan. Forest Ecol Manage 140:163–175.

Bellingham PJ, Kohyama T, Aiba S (1996) The effects of a typhoon on Japanese warm- temperate rain forests. Ecol Res 11:229–247.

Kohyama T (1988) A function describing all-sized trunk diameter distribution in warm-temperate rain forests. Bot Mag Tokyo 101:207–212.

Kohyama T (1989) Simulation of the structural development of warm-temperate rain forest stands. Ann Bot 63:625–634.

Kohyama T, Aiba S (1997) Dynamics of primary and secondary warm-temperate rain forests in Yakushima Islands. Tropics 6:383–392.

Magurran AE (2004) Measuring biological diversity. Blackwell, Malden.

Sakai A, Hirayama T, Oshioka S, Hirata Y (2006) Effects of elevation and postharvest disturbance on the composition of vegetation after the clear-cut harvest of conifer plantations in southern Shikoku, Japan. J For Res 11:253–265.

ter Braak CJF, Smilauer P (2002) CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5). Microcomputer Power, Ithaca.

Tsujino R, Yumoto T (2004) Effects of sika deer on tree seedlings in a warm temperate forest on Yakushima Island, Japan. Ecol Res 19:291–300.

Tsujino R, Hino T, Agetsuma N, Yumoto T (2006) Variation in tree growth, mortality and recruitment among topographic positions in a warm temperate forest. J Veg Sci 17:281–290.

Yamagawa H, Ito S (2006) The role of different sources of tree regeneration in the initial stages of natural forest recovery after logging of conifer plantation in a warm-temperate region. J For Res 11:455–460.

Effects of Sika Deer and Conifer Plantations on the Density and Diversity of Current-Year Tree Seedlings in Lowland Forests on Yakushima Island, Japan

Riyou Tsujino[1], **Naoki Agetsuma**[2], **Yoshimi Agetsuma-Yanagihara**[3]
[1]*Research Institute for Humanity and Nature, 457-4 Motoyama, Kamigamo, Kita-ku, Kyoto, Japan*
[2]*Tomakomai Experimental Forest, Field Science Center for Northern Biosphere, Hokkaido University, Takaoka, Tomakomai, Hokkaido, Japan*
[3]*Association of Tomakomai City Museum, 3-9-7 Suehiro, Tomakomai, Hokkaido, Japan*

Introduction

Sika deer populations have dramatically increased recently, producing increasing damage to forest vegetation in several areas in Japan (Takatsuki 1989; Takatsuki and Gorai 1994; Akashi and Nakashizuka 1999; Yokoyama et al. 2001; Tsujino et al. 2004). Grazing and browsing by high-density sika deer populations cause a loss of forest floor vegetation (Takatsuki and Gorai 1994; Tsujino and Yumoto 2004), a decrease of mature trees owing to bark stripping (Akashi and Nakashizuka 1999; Yokoyama et al. 2001), and an increase of some special plant species, which are not preferred by sika deer (Kaji and Yajima 1991). Large-scale logging and planting of commercial crops have also damaged ecological functions of forests and have eroded the public benefit provided by forests (Agetsuma 2007).

We should note first that most natural vegetation has already been disturbed by forest transformation (Agetsuma 2007). Thus in this study, we examined vegetation changes in the forest in relation to sika deer herbivory and human impacts such as conifer plantations. To clarify the effects of sika deer and conifer plantations on changes of forest vegetation, we established deer exclusion fences in three study sites, which showed differences in sika deer population density and percentage of area occupied by conifer plantations, and counted newly germinated and established current-year tree seedlings in summer and winter.

Methods

The study sites were located on Yakushima Island (30˚20' N, 131˚30' E), which has an area of ca. 503 km^2 and lies ca. 70 km south of Kyushu, Japan. The study area was in a warm-temperate forest at an altitude between 200 and 600 m on the western and northwestern part of Yakushima Island. The geology is granite. The forest consists mainly of broad-leaved evergreen trees of Fagaceae, Hamamelidaceae, Myrsinaceae, and Lauraceae (Tagawa 1980; Tsujino et al. 2006; Agetsuma et al. unpubl. data).

Three study sites with differences in the percentage of plantation area and sika deer population density were selected: the Seibu area (SE, ca. 250 m asl, Fig. 1), the Nagata area (NA, ca. 550 m asl, Fig. 1), and the Isso area (IS, ca. 500 m asl, Fig. 1). The estimated sika deer population density was greatest in SE and lowest in IS (Agetsuma et al. 2003; Agetsuma 2007), and the disturbance ranking, calculated as the percentage of total area occupied by conifer plantations, was IS > NA > SE (Hill et al. 1994).

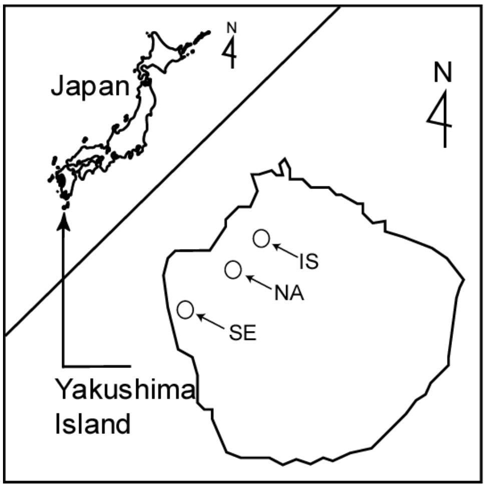


Fig. 1. Study sites on Yakushima Island, Japan. IS, NA, and SE indicate the Isso area (500 m asl), Nagata area (550 m asl), and Seibu area (250 m asl), respectively.

A subspecies of sika deer, *Cervus nippon yakushimae*, inhabits the whole area of Yakushima Island (Kagoshimaken Sizenaigo Kyokai 1981). The body weights of adult male and female *C. n. yakushimae* are 24–50 kg and 19–25 kg, respectively (Kagoshimaken Sizenaigo Kyokai 1994; Agetsuma et al. 2003; Komiya 2002). The deer population density on Yakushima Island may vary according to altitude, forest type, and degree of human impact. Natural predators of deer are absent. Deer were hunted in some areas on the island that suffered substantial damage to agriculture, but not in the study area. In broad-leaved evergreen forests in Japan, the leaves and fruits of evergreen and deciduous plants and graminoids are the primary food for sika deer (Takatsuki 1988; Takatsuki 1990; Asada and Ochiai 1996; Tsujino and Yumoto 2004; Agetsuma and Agetsuma-Yanagihara 2006).

Table 1. Numbers of seedling observation quadrats(1-m^2) at the three study sites.

	Number of Quadrats		
Study Site	Fenced	Unfenced	Total
Seibu Area	27	27	54
Nagata Area	17	22	39
Isso Area	22	22	44
Total	66	71	137

Six deer exclusion fences, each with a perimeter of ca. 80 m and an area of ca. 0.02 ha, were established and were duplicated at each site, at the Seibu and Isso areas in August 2003 and at the Nagata area in August 2004 (Table 1). An unfenced area of similar size was also selected in the vicinity of each fenced area. These study quadrats were divided into sub-quadrats of 5 × 5 m, and a total of 137 seedling observation quadrats (1 × 1 m) were established at the southwest corner of each sub-quadrat in fenced and unfenced quadrats in December 2003 (Table 1).

A seedling census was conducted for all living current-year tree seedlings in early summer (late May to early July) and winter (December to February). Most tree seedlings in this area germinated from April to June; the seedling growing season was summer to autumn; and seedling growth and mortality decreased in winter.

Thus, we defined current-year tree seedlings as seedlings less than 1 year old. We counted the number of current-year tree seedlings for each tree species in summer and winter, to compare seedling recruitment and establishment between fenced and unfenced quadrats and among study sites. We collected data four times at SE and IS in winter, three times at SE and IS in summer, three times at NA in winter, and two times at NA in summer. Thus, the replication numbers were 3 for SE, 2 for NA, and 3 for IS in summer, and 4 for SE, 3 for NA, and 4 for IS in winter.

A generalized linear model with a stepwise procedure was used to assess the relationship among the number of current-year tree seedlings (NSE), number of species (NSP), census season (SEA), study site (SIT), and fence treatment (FEN), starting from a full model with interactions:

Model 1

NSE ~ SEA + SIT + FEN + SEA:SIT + SEA:FEN + SIT:FEN + SEA:SIT:FEN

Model 2

NSP ~ SEA + SIT + FEN + SEA:SIT + SEA:FEN + SIT:FEN + SEA:SIT:FEN

where SEA (summer or winter), SIT (Seibu, Nagata, or Isso area), and FEN (fenced or unfenced quadrats) are categorical variables with two or three modalities. The colon indicates an interaction. We used Akaike's information criterion (AIC, Akaike 1973) for the model selection, with the minimum AIC as the best-fit estimator.

Results and discussion

A statistical analysis of the number of current-year tree seedlings selected the full model of the regression as that with the minimum AIC value (AIC = 4923.24). In both summer and winter, the mean number of seedlings per quadrat was greater in fenced quadrats than in unfenced quadrats (Fig. 2). In NA and SE, the high-density sika deer population probably grazed on newly germinated seedlings soon after germination, and the difference in seedling density between fenced and unfenced quadrats was great, especially in summer. On the other hand, newly germinated seedlings in IS were not browsed as much owing to the low density of sika deer in IS, and the difference in seedling density between fenced and unfenced quadrats was small. In winter, seedling establishment numbers were different between fenced and unfenced quadrats at each study site, probably because of feeding pressure and physical disturbance by sika deer.

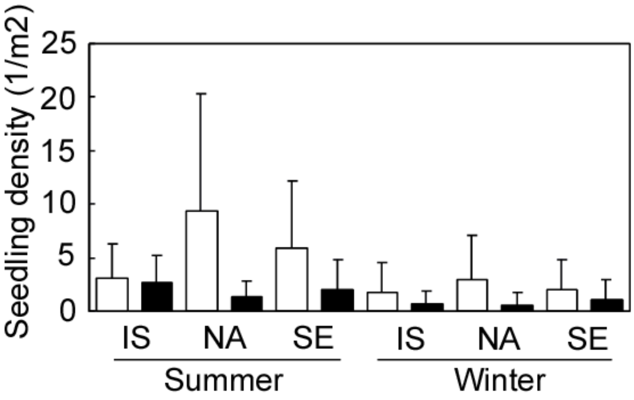


Fig. 2. Mean (mean + SD, white and black bars) numbers of current-year tree seedlings per 1-m^2 quadrat. White and black indicate fenced and unfenced quadrats, respectively. IS, NA, and SE indicate the Isso area, Nagata area, and Seibu area, respectively.

To identify the effects of sika deer and human impacts on tree seedling diversity in the forest, we compared the number of current-year tree seedling species among sites with different sika deer density and disturbance intensity. A statistical analysis of the number of species selected the full model with the minimum AIC value (AIC = 2896.95) (Fig. 3). The mean number of species per fenced quadrat was estimated to be greater at SE > NA > IS in summer and at NA > SE > IS in winter, and the difference between fenced and unfenced quadrats was estimated to be greater at SE > NA > IS in summer and NA > SE > IS in winter. This result indicates that human impacts, such as plantation areas, around the study sites limited the recruitment and establishment of current-year tree seedlings, especially in IS, probably because the conversion to conifer plantations decreased the natural forest area of seed sources. On the other hand, the mean number of species per unfenced quadrat was greater at IS > SE > NA in summer and in winter, with the difference between fenced and unfenced as above (SE > NA > IS in summer and NA > SE > IS in winter). This indicates that the feeding and/or physical disturbance owing to sika deer (Tsujino and Yumoto 2004) caused the decrease of seedling recruitment and establishment in unfenced quadrats, especially in SE and NA, and resulted in decreased seedling diversity.

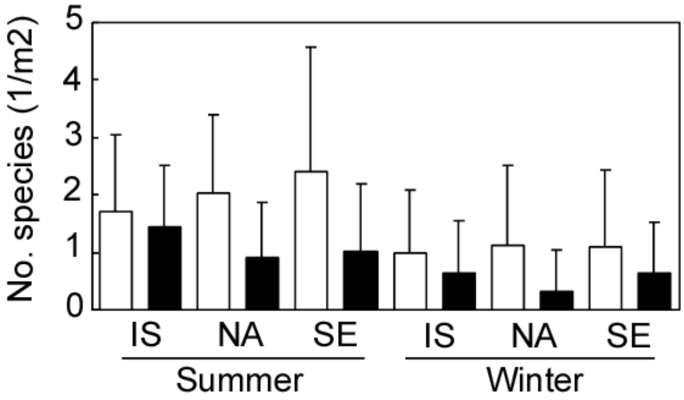


Fig. 3. Mean (mean + SD, white and black bars) number of species of current-year tree seedlings per 1-m^2 seedling observation quadrat. White and black indicate fenced and unfenced quadrats, respectively. IS, NA, and SE indicate the Isso area, Nagata area, and Seibu area, respectively.

In this study, we revealed two factors affecting tree seedling number and diversity: sika deer populations negatively affect both the number and diversity of current-year tree seedlings, and conifer plantations negatively affect the diversity of seedlings (Fig. 4). On the other hand, there is a negative relationship between sika deer population density and degree of human impact on the forest (Agetsuma 2007). We suggest that human impacts on a forest, such as conversion of natural forest to conifer plantation, have not only direct negative effects on tree seedling diversity, probably through a seed source effect, but also indirect positive effects by decreasing the sika deer density (Fig. 4). Thus, the effects of forest disturbances by humans are complex and include direct negative and indirect positive effects on tree seedling communities.

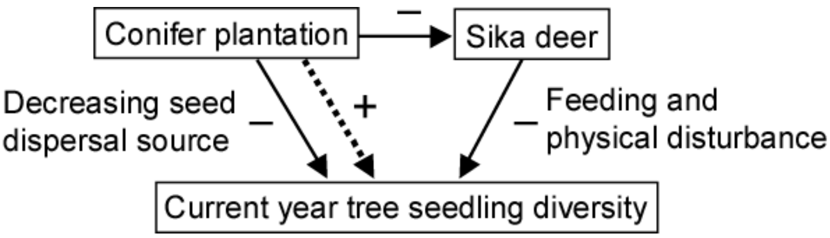


Fig. 4. Scheme showing the interactions among conifer plantations, sika deer, and current-year tree seedling diversity. Solid and broken lines indicate direct and indirect effects, respectively. Plus and minus signs beside arrows indicate positive and negative effects, respectively.

References

Agetsuma N, Sugiura H, Hill DA, Agetsuma-Yanagihara Y, Tanaka T. (2003) Population density and group composition of Japanese sika deer (*Cervus nippon yakushimae*) in ever-green broad leaved forest of Yakushima, southern Japan. Ecol Res 18: 475-483.

Agetsuma N. (2007) Ecological function losses caused by monotonous land use induce crop raiding by wildlife on the island of Yakushima, southern Japan. Ecol Res 22: 390-402.

Agetsuma N, Agetsuma-Yanagihara Y. (2006) Ecology of Yaku sika deer in the island of Yakushima (in Japanese). In: Ohsawa M, Tagawa H, Yamagiwa J (eds) World heritage, Yakushima. Asakura, Tokyo, pp 143–149.

Akashi N, Nakashizuka T.（1999） Effects of bark-stripping by Sika deer（*Cervus nippon*）on population dynamics of a mixed forest in Japan. Forest Ecol Manag 113: 75-82.

Asada, M. and Ochiai, K. (1996) Food habitats of sika deer on the Boso Peninsula, central Japan. Ecological Research 11: 89-95.

Hill DA, Agetsuma N, Suzuki S. (1994) Preliminary survey of group density of *Macaca fuscata yakui* in relation to logging history at seven sites in Yakushima Japan. Primate Research 10: 85-93.

Kagoshimaken Sizenaigo Kyokai. (1981) Yakusika no seisoku/bunnpu nikannsuru kinkyu chousa houkokusho. Kagoshimaken Sizenaigo Kyokai Chousahoukoku 5: 1-34 (in Japanese).

Kagoshimaken Sizenaigo Kyokai (1994) ‘Kendo Kamiyaku-Nagata-Yakusen Kairyoukeikaku Nitomonau Kankyoeikyo Monitoring’ Houkokusho. Kagoshimaken Sizenaigo Kyokai, Kagoshima (in Japanese).

Kaji K, Yajima T. (1991) Forage selection by introduced deer on Nakanoshima Island, and its effect on the forest vegetation. Proceedings of the International Symposium on Wildlife Conservation, in INTECOL 1990（ed. N.Maruyama *et al.*） pp. 52-55. Japan Wildlife Research Center, Tokyo.

Komiya T. (2002) Field Best Encyclopedia: Mammals in Japan. Gakken, Tokyo (In Japanese).

Takatsuki S. (1988) Rumen contents of sika deer on Tsushima Island, western Japan. Ecological Research 3: 181-183.

Takatsuki S. (1989) Effect of deer on plants and plant communities. Japanese Journal of Ecology 39: 67-80 (In Japanese).

Takatsuki S. (1990) Summer dietary compositions of sake deer on Yakushima Island southern Japan. Ecological Research 5: 253-260.

Takatsuki S, Gorai T.（1994） Effects of Sika deer on the regeneration of a *Fagus crenata* forest on Kinkazan Island, northern Japan. Ecological Research 9: 115-120.

Tsujino R, Yumoto T. (2004) Effects of sika deer on tree seedlings in a warm temperate forest on Yakushima Island, Japan. Ecological Research 19: 291-300.

Tsujino R, Noma N, Yumoto T. (2004) Growth of sika deer (*Cervus nippon yakushimae*) population in the lowland forests of Yakushima Island, Japan. Mammal Study 25: 105-111.

Tsujino R, Takafumi H, Agetsuma N, Yumoto T. (2006) Variation in tree growth, mortality and recruitment among topographic positions in a warm–temperate forest. Journal of Vegetation Science 17: 281-290.

Yokoyama S, Maeji I, Ueda T, Ando M, Shibata E.（2001） Impact of bark stripping by sika deer, *Cervus nippon*, on subalpine coniferous forests in central Japan. Forest Ecology and Management 140: 93-99.

Changes in Insect Assemblages with Conversion from Old-Growth Evergreen Broadleaf Forests to *Cryptomeria japonica* Plantations on Yakushima Island, Japan

Takeo Yamauchi[1], Shin-ichiro Aiba[2], Riyou Tsujino[3], Takakazu Yumoto[3]
[1] *Toyama Institute of Health, Imizu, Toyama 939-0363, Japan.*
[2] *Faculty of Science, Kagoshima University, Kagoshima 890-0065, Japan.*
[3] *Research Institute for Humanity and Nature, Kyoto 603-8047, Japan.*

Introduction

Recently, several studies have investigated the changes in insect assemblages after conversion from natural or secondary forests to plantations of conifers (primarily Japanese cedar, *Cryptomeria japonica*, and hinoki cypress, *Chamaecyparis obtusa*) in the temperate deciduous forests of Japan (e.g., Inada et al., 2006; Maeto & Makihara, 1999; Makino et al., 2007; Sayama et al., 2005; Sueyoshi et al., 2003). However, changes in insect communities in systems other than temperate deciduous forests in Japan have been less thoroughly examined (but see Maeto et al., 2002; Mizota & Imasaka, 1997). Thus, there is little information regarding shifts in insect assemblages in response to the conversion of evergreen broadleaf forests in Japan.

We examined the responses of insect assemblages to the conversion of an evergreen broadleaf forest to a *C. japonica* plantation. We compared the abundance and diversity of seven insect groups (tiger beetles, clerid beetles, longicorn beetles, leaf beetles, elephant beetles other than Curculionidae, hoverflies, and wasps) collected in old-growth evergreen broadleaf forests and *C. japonica* plantations in the lowlands on Yakushima Island, Japan.

Materials & Methods

1) Study sites

The study sites were located in lowland forests (150-250 m above sea level) on Yakushima Island, Kagoshima Prefecture, Japan. We selected two plantations of *C. japonica* (approximately 40 years old) in each of the eastern and western regions of the island and two old-growth evergreen broadleaf forests situated near the two plantations (Table 1). Each study site was located within a continuous stand at least 100 m from the boundaries of other forest types, although small patches of secondary stands of broadleaf forests were occasionally found in the vicinity of the plantations. Both old-growth sites are located in a National Forest, whereas both plantations are under private ownership. The forest floors of the plantations receive more light due to the process of tree thinning.

2) Sampling and identification

Townes-type, white Malaise traps (1.8 x 1.8 x 1.8 m) were used to capture insects. Within each site, three traps were haphazardly set, and the maximum distance between traps was < 100 m. Trapped insects were collected monthly from July to December 2006 and from March to May 2007. A mixture of 70 % ethanol and ethylene glycol was added as a preservative to the insect containers in the traps. Collected insects were morphologically identified to species.

3) Target insect groups

Five coleopteran groups [Tiger beetles (Cicindelidae), clerid beetles (Cleridae), longicorn beetles (Disteniidae and Cerambycidae), leaf beetles (Chrysomelidae), and elephant beetles other than Curculionidae (Anthribidae, Brentidae, and Rhynchophoridae)], one dipteran group [hoverflies (Syrphidae)], and one hymenopteran group [wasps (Vespidae)] were examined. These insect groups consist of several functional feeding types. Tiger beetles, clerid beetles, and wasps are predators, whereas longicorn beetles, elephant beetles, and leaf beetles are herbivores. Adult hoverflies are pollen feeders (herbivores), and the larvae consist of two feeding types in the species examined: members of the subfamily Syrphinae are aphid-feeders (predators), and members of the subfamily Eristalinae are decayed-wood feeders (herbivores). We based guild assignment of hoverflies on larval feeding habits.

4) Data analyses

Data for each taxonomic group were pooled for each site throughout the season, and species with sample sizes > 20 individuals were analyzed using chi-squared tests. Data were compared between the two forest types (all individuals collected in old-growth evergreen broadleaf forests versus *C. japonica* plantations). Expected values were calculated by multiplying the number of total captures for each species by 0.5, because the number of individuals of all target species combined was nearly identical between the two forest types.

We calculated the logarithm of the inverse Simpson's index as an index of species diversity, as follows:

$$\log (1/D) = \log \Sigma \ [N (N-1)] / [Ni (Ni-1)]$$

where N is the total number of individuals, and Ni is the number of individuals of the ith species.

Results & Discussion

We collected 431 individuals of 51 species of target insect groups in the *C. japonica* plantations and 447 individuals of 76 species in the old-growth evergreen broadleaf forests (Table 2). Indices of species diversity calculated for each insect group were lower in the *C. japonica* plantations than in the old-growth evergreen broadleaf forests (Table 2).

The total number of herbivorous species was much greater in the old-growth evergreen broadleaf forests (58 species) than in the *C. japonica* plantations (30 species). Herbivores depend directly on a wide variety of plants for food and shelter (Siemann, 1998). Therefore, the greater availability of more plant species in the old-growth evergreen broadleaf forests likely increased the species diversity of herbivores. In contrast, the total number of predator species did not significantly differ between the *C. japonica* plantation (21 species) and the old-growth evergreen broadleaf forest (18 species). These results suggest that the species diversity of predators was not greatly affected by the increased availability of plants in the old-growth evergreen broadleaf forest, because insect predators do not directly depend on a variety plants for food.

The numbers of trapped individuals of *Cicindela japonica*, *Stigmatium nakanei*, *Omadius nigromaculatus*, *Ceresium longicorne*, *Cryptocephalus perelegans*, and *Monolepta pallidula* were significantly greater in the old-growth evergreen broadleaf forest than in the *C. japonica* plantations (Tables 3-6). The recorded host plants of *C. longicorne* and *C. perelegans* are primarily broadleaf trees and do not include Japanese cedar (Kimoto and Takizawa, 1994; Takeda, 2007). These six species include three predators, *C. japonica*, *S. nakanei*, and *O. nigromaculatus*; thus, some predators may indeed be affected by the conversion of old-growth evergreen broadleaf forests to *C. japonica* plantations.

In contrast, the number of trapped individuals of Alticinae gen. sp., *Sipalinus gigas*, *Episyrphus balteatus*, and *Chalcosyrphus frontalis* were significantly greater in the *C. japonica* plantations than in the old-growth evergreen broadleaf forest (Tables 6-8). In addition, *Milesia oshimaensis* (28 individuals) were collected only in the *C. japonica* plantations (Table 8). Although the host plants of Alticinae gen. sp. and *C. frontalis* are unknown, the recorded host plants of *S. gigas* and *M. oshimaensis* include Japanese cedar (Ikezaki, 1976; Morimoto, 1984). *Episyrphus balteatus* is a predator that may indirectly depend on *C. japonica*.

As has been previously recorded in temperate deciduous forests (e.g., Makino et al., 2007), our results indicate that the conversion of evergreen broadleaf forests to *C. japonica* plantations causes shifts in insect assemblages. Such conversions may lead to the impoverishment of insect herbivores. Insect predators may also be affected by the disappearance of evergreen broadleaf forests. However, several insect species were rarely collected in the evergreen broadleaf forests and may appear and/or increase explosively in the *C. japonica* plantations.

References

Ikezaki Y (1976) Syrphidae from Tsushima. In: Nagasaki Biological Society (ed) Tsushima no Seibutsu (in Japanese). Nagasaki Biological Society, Nagasaki, pp 329-339

Inada T, Yunomura S, Maeto K (2006) The influence of forest management on the assemblage of longicorn beetles (in Japanese with English summary). J Jpn For Soc 88:446-455

Kimoto S, Takizawa H (1994) Leaf beetles (Chrysomelidae) of Japan (in Japanese with English summary). Tokai University Press, Tokyo

Maeto K, Makihara H (1999) Changes in insect assemblages with secondary succession of temperate deciduous forests after clear-cutting (in Japanese with English summary). Jpn J Entomol (NS) 2:11-16

Maeto K, Sato S, Miyata H (2002) Species diversity of longicorn beetles in humid warm-temperate forests: the impact of forest management practices on old-growth forest species in southwestern Japan. Biodiv Conserv 11:1919-1937

Makino S, Goto H, Hasegawa M, Okabe K, Tanaka H, Inoue T, Okochi I (2007) Degradation of longicorn beetle (Coleoptera, Cerambycidae, Disteniidae) fauna caused by conversion from broad-leaved to man-made conifer stands of *Cryptomeria japonica* (Taxodiaceae) in central Japan. Ecol Res 22:372-381

Mizota K, Imasaka S (1997) Comparison of flower-visiting beetle communities between natural and artificial forests in southern Kii Peninsula (in Japanese with English summary). Res Bull Hokkaido Univ For 54: 299-326

Morimoto K (1984) Rhynchophoridae. In Hayashi M (ed) The Coleoptera of Japan in color Vol. IV (in Japanese). Hoikusha, Osaka, pp 345-348

Sayama K, Makihara H, Inoue T, Okochi I (2005) Monitoring longicorn beetles in different forest types using collision traps baited with chemical attractants (in Japanese with English summary). Bull FFPRI 4:189-199

Siemann E (1998) Experimental tests of effects of plant productivity and diversity on grassland arthropod diversity. Ecology 79:2057-2070

Sueyoshi M, Maeto K, Makihara H, Makino S, Iwai T (2003) Changes in dipteran assemblages with secondary succession of temperate deciduous forests following clear-cutting (in Japanese with English summary). Bull FFPRI 2: 171-191

Takeda M (2007) Host plants. In: Ohbayashi N, Niisato T (eds) Longicorn beetles of Japan (in Japanese). Tokai University Press, Tokyo, pp 683-773

Table 1 Location and forest type of the investigated sites in Yakushima Island

Site	Forest type	Site position	Altitude (m)
CE	Plantation of *C. japonica* (40 yr.)	Eastern part	150
CW	Plantation of *C. japonica* (40 yr.)	Western part	220
OE	Old-growth evergreen broadleaf forest	Eastern part	170
OW	Old-growth evergreen broadleaf forest	Western part	250

Table 2 Comparisons of number of species, number of individuals, and diversity indices [logarithm of inverse Simpson's index, log(1/D)] between plantation of cedar and old-growth evergreen broadleaf forest in Yakushima

	Plantation of cedar			Old-growth evergreen broadleaf forest		
	No. of species	No. of individuals	log(1/*D*)	No. of species	No. of individuals	log(1/*D*)
Tiger beetles	1	5	0.00	2	47	0.20
Clerid beetles	5	35	0.31	7	98	0.40
Longicorn beetles	8	17	0.92	26	94	0.96
Leaf beetles	8	145	0.12	12	123	0.76
Elephant beetles	7	40	0.22	16	39	1.07
Hoverflies	15	170	0.59	7	21	0.80
Wasps	7	19	0.20	6	25	0.47
All target groups combined	51	431	0.87	76	447	1.34

Table 3 The number of tiger beetles collected with 3 Malaise traps in Yakushima

	CE	CW	OE	OW	Total	χ^2 (CE+CW *vs* OE+OW)
Cicindelidae						
Cicindela japonica	2	3	9	27	41	23.43*
Therates alboobliquatus			1	10	11	

*$P<0.001$.

Table 4 The number of clerid beetles collected with 3 Malaise traps in Yakushima

	CE	CW	OE	OW	Total	χ^2 (CE+CW *vs* OE+OW)
Cleridae						
Tillus igarashii				1	1	
Opilo carinatus		1	1	8	10	
Clerus postmaculatus				3	3	
Stigmatium pilosellum				1	1	
Stigmatium nakanei	2	22	25	34	83	14.75*
Stigmatium igai		2	1	7	10	
Omadius nigromaculatus		6	16	1	23	5.26**
Allochotes dichrous		2			2	

*$P<0.001$; **$P<0.05$.

Table 5 The number of longicorn beetles collected with 3 Malaise traps in Yakushima

	CE	CW	OE	OW	Total	χ^2 (CE+CW *vs* OE+OW)
Disteniidae						
Distenia gracilis gracilis				1	1	
Cerambycidae						
Lepturinae						
Leptura ochraceofasciata ochraceofasciata		1	1		2	
Cerambycinae						
Allotraeus sphaerioninus			2		2	
Stenodryas clavigera clavigera				1	1	
?*Ceresium holophaeum*				6	6	
Ceresium longicorne		3		25	28	17.28＊
Ceresium sp.				2	2	
Comusia testacea				1	1	
Stenhomalus nagaoi				1	1	
Artimpaza setigera japonica				1	1	
Cleomenes takiguchii				4	4	
Callidiellum rufipenne	2				2	
Xylotrechus emaciatus		4	10	5	19	
Perissus kiusiuensis kiusiuensis			1	3	4	
Chlorophorus quiquefasciatus	1		2	1	4	
Chlorophorus muscosus			1	4	5	
Demonax transillis				2	2	
Anaglyptus arakawae kumagensis			2		2	
Clytini gen. sp.			1		1	
Lamiinae						
Mesosa longipennis			1		1	
Neosybra cribrella			1		1	
Sybra ordinata ordinata				3	3	
Pterolophia gibbosipennis subcristipennis				3	3	
?*Pterolophia kyushuensis*			1	1	2	
Uraecha bimaculata	1		2	2	5	
Xenicotela pardalina		1			1	
Mimectatina meridiana ohirai	1	3			4	
Rondibilis insularis			1		1	
Sciades fasciatus fasciatus				2	2	

＊$P<0.001$

Table 6 The number of leaf beetles collected with 3 Malaise traps in Yakushima

	CE	CW	OE	OW	Total	χ^2 (CE+CW *vs* OE+OW)
Chrysomelidae						
Cryptocephalinae						
Cryptocephalus perelegans		1	5	21	27	23.14*
Eumolpinae						
Acrothinium gaschkevitchii			5	8	13	
Basilepta hirayamai		1	4	5	10	
Trichochrysea okinawana				1	1	
Lypesthes fulvus				7	7	
?*Lypesthes itoi*	9	3	1		13	
Hyperaxis fasciata		2	2	5	9	
Demotina fasciculata			1		1	
Demotina sp.			13	1	14	
Galerucinae						
Fleutiauxia armata		1			1	
?*Monolepta pallidula*		1	9	30	40	36.10*
Alticinae						
Nonarthra cyanea				1	1	
?*Nonarthra tibialis*	1				1	
Alticinae gen. sp.	114	12		4	130	114.49*

*P＜0.001.

Table 7 The number of elephant beetles (excluging Curculionidae) collected with 3 Malaise traps in Yakushima

	CE	CW	OE	OW	Total	χ^2 (CE+CW *vs* OE+OW)
Anthribidae						
Ozotomerus nigromaculatus				1	1	
Mecotropis kyushuensis			1		1	
Stiboderes impressus stibinus	1				1	
?*Dendrotrogus japonicus*			2		2	
Sintor bipunctatus			2		2	
Sympaector rugirostris			1		1	
Acorynus asanoi		2	5	3	10	
Litocerus kimurai				1	1	
Litocerus tokarensis insensibilis			1	1	2	
Litocerus multiguttatus		1		1	2	
?*Phaulimia confinis*				1	1	
Phaulimia aberrans				2	2	
Oxyderes fastigatus		1	2	3	6	
Aphaulimia debilis		1	2	4	7	
Habrissus unciferoides		3	2	2	7	
Brentidae						
Baryrhynchus poweri				1	1	
Rhynchophoridae						
Sipalinus gigas	31			1	32	28.12*

*P＜0.001.

Table 8 The number of hoverflies collected with 3 Malaise traps in Yakushima

	CE	CW	OE	OW	Total	χ^2 (CE+CW *vs* OE+OW)
Syrphidae						
Syrphinae						
?*Allograpta iavana*				1	1	
Didea fasciata		1			1	
Episyrphus balteatus	42	25	3	4	74	48.64*
?*Ischiodon scutellaris*				1	1	
?*Parasyrphus aeneostoma*		1			1	
?*Syrphus torvus*	1				1	
Allobaccha nubilipennis	3	3			6	
Baccha maculata		1			1	
Chrysotoxum sp.	1				1	
Platycheirus sp.	2	2			4	
Eristalinae						
?*Eumerus japonicus*		1	1	3	5	
?*Mallota yakushimana*		1			1	
Milesia oshimaensis	18	6			24	
Milesia undulata	1		3		4	
Takaomyia johannis		5	1	1	7	
Chalcosyrphus frontalis	25	23	3		51	39.70*
Xylota sp.	6	2			8	

*$P<0.001$.

Table 9 The number of wasps collected with 3 Malaise traps in Yakushima

	CE	CW	OE	OW	Total	χ^2 (CE+CW *vs* OE+OW)
Vespidae						
Polistes rothneyi	1				1	
Parapolybia indica	1	6			7	
Vespa mandarinia	2			1	3	
Vespa ducalis	2	1	1		4	
Vespa analis	1	2		1	4	
Vespa similima	1	1	3	9	14	
Vespula flaviceps			1		1	
Vespula shidai		1		9	10	

Comparison of Food Availability and the Density of Japanese Macaques in Primary, Naturally Regenerated, and Plantation Forests

Goro Hanya[1], Koichiro Zamma[1], Shuhei Hayaishi[2], Shinichi Yoshihiro[3], Yosuke Tsuriya[4], Shuji Sugaya[5], Masahiro M. Kanaoka[2], Sachiko Hayakawa[1], Yukio Takahata[6]
[1]*Primate Research Institute, Kyoto University, Inuyama, Japan*
[2]*Graduate School of Science, Kyoto University, Kyoto, Japan*
[3]*Ryukoku University, Kyoto, Japan*
[4]*Nihon University, Fujisawa, Japan*
[5]*Higashino, Kasugai, Japan*
[6]*Kwansei-gakuin University, Sanda, Japan*

Introduction

Japanese forests have changed considerably since the 1960s through ‘expansive afforestation,’ that is, through deforestation and replacement of broad-leaved trees with conifers, such as Japanese cedar *Cryptomeria japonica* and hinoki cypress *Chamaecyparis obtusa*. As a result, about 40% of Japanese forests have been converted to artificial coniferous forests. These tree species do not provide food for most indigenous vertebrate species, and are therefore believed to significantly affect Japanese wildlife. For example, crop raiding by Japanese macaques *Macaca fuscata* rapidly increased in the 1980s, and the area of damaged farmland has now reached 6000 ha. Agetsuma (1998) speculated that for about 10 yr after logging, food availability for the macaques did not decline because they foraged the grass vegetation in logged patches. However, as planted conifer stands developed, the grass was gradually replaced by tree plantations. In the 10–20 yr after logging, the planted forest became an artificial coniferous forest that provided little food for the macaques, leading them to raid crops. Damage to crops and forestry products by black bears (*Ursus thibetanus*), sika deer (*Cervus nippon*), Japanese serow (*Capricornis crispus*), Japanese hares (*Lepus brachyurus*), and wild boars (*Sus scrofa*) are also serious problems, which are suspected to be caused directly or indirectly by expansive afforestation (Takatsuki, 1996). In the 1990s, about 8000 Japanese macaques, 1200 Japanese serow, and 1500 black bears were killed annually as pests in Japan, even though some of these populations were endangered. Thus, to reduce damage to crops and to conserve wildlife, it is important to clarify and quantify the effects of expansive afforestation. In recent years, large-scale expansive afforestation has rarely been practiced due to the lack of adequate labor, and logged broad-leaved forests have often regenerated naturally with minimal human management. However, little empirical data exist on the disparate effects of these two regeneration systems on large, forest-dwelling mammals in Japan.

We compared food availability and group density of Japanese macaques on Yakushima Island, Japan, in three different forest types: primary forest, naturally regenerating forest, and artificially regenerating forest planted with *Cryptomeria japonica*. On this island, as in other parts of Japan, large-scale expansive afforestation was conducted in the 1960s and 1970s, and crop raiding by Japanese macaques became a serious problem in the 1980s. Now the annual cost of damage to crops is 10–40 million yen and more than 400 macaques are killed annually on Yakushima for pest control (Agetsuma, 1998). The goal of this study was to clarify differences among these forests types with respect to food availability and the density of Japanese macaques.

Methods

The study site was located in the western part of Yakushima at 700–1200 m above sea level. The primary vegetation here is transitional between a higher elevation coniferous forest and a lower elevation warm–temperate broad-leaved evergreen forest. The study area included both primary forest protected as national park and disturbed forest outside the park (Fig. 1a). The study in 2003 was conducted 7–27 yr after logging. For simplicity, we hereafter refer to the age of the regenerating forests in relation to the year 2003. The regeneration system changed in 1984. Before 1984, expansive afforestation was conducted: the forest was clear-cut, the felled areas were cleared, and 1000–2000 conifer saplings (*Cryptomeria japonica*) about 60 cm tall were planted per hectare. Weeding was conducted once a year for a few years after logging. Since 1984, no afforestation has occurred and clear-cut forests have been left to regenerate with little human management. We refer to the former stands as ‘plantation’ and the latter as ‘naturally regenerated.’

We established 5 × 5-m plots in primary and logged forests. In the primary forest, plots were randomly selected within a larger (0.75 ha) plot established in 1999. In the logged forest, we set each plot at the approximate center of each logged patch in 2002. We established 30 plots in primary forest, 10 in plantation forest (five in 19–22-yr-old and five in 23–27-yr-old forests) and 17 in naturally regenerated forest (seven in 7–10-yr-old, five in 11–13-yr-old, and five in 14–18-yr-old forests). In each plot, we recorded the species and diameter at breast height (DBH) of all trees taller than 1 m.

In early September of 2002 and 2003, we studied the fruit production of species eaten by macaques. This was carried out by a single observer counting the number of fleshy fruits on marked branches of each tree in each plot. The number of fruits in the tree was estimated by dividing the number of fruits on marked branches by the proportion of the branch relative to the total crown.

We censused Japanese macaques in August 2000, 2001, 2002, and 2003 using a point census with group follows (Hanya et al., 2003). We divided the 7.5-km^2 census area into 30 500 × 500-m grid squares (Fig. 1b). One observer was positioned in each grid square at a fixed point. Observers stayed at the points from 06:00 to 16:00. When they detected macaques by direct observation or vocalization, they recorded the time and approximate location, regardless of whether the animals were within or outside of the grid square. Whenever possible, 10–14 researchers followed groups that appeared in the census area. Group followers recorded the location of the center of the group every hour. Each point was censused for 6 days in 2000, 9 days in 2001, 7 days in 2002, and 5 days in 2003.

To estimate group density, we defined a group as a cluster of macaques whose spread was 500 m at maximum. At each point, we counted the number of groups detected each hour (e.g., 06:00–06:59) as follows. The number of groups detected each hour was averaged for each day, and then the day’s average value was averaged across all census days of the year for each point. We refer to this value, *n*, as the number of groups detected at a point. Because we followed some groups, it was possible to calculate the proportion of groups detected by point observers (detectability) at a given distance. We regressed the relationship between point-group distance y and detectability g(y) on a half-normal model: $g(y) = e^{-\lambda y^2}$ and calculated λ as the 'detectability constant.' Then, group density D was calculated as $D = \frac{\lambda n}{\pi}$ (Hanya et al., 2003).

Results

Fruit production was higher in young naturally regenerated forests than in primary forests (Fig. 2). However, the pattern varied among the four main fleshy-fruited species (Fig. 3), which comprised more than 95% of the fruit production. Fruit production was highest in 11–13-yr-old forests for *Eurya japonica* and 11–13-yr-old forests for *Symplocos myrtacea*. In the case of *Symplocos tanakae*, fruit production did not vary between primary and naturally regenerated forests in 2002 and was largely restricted to the 11–13-yr-old naturally regenerated forest in 2003. In contrast, *Cleyera japonica* fruited only in primary forest.

The density of macaque groups was higher in the youngest (7–10-yr-old) naturally generated forest than in the oldest (23–27-yr-old) plantation forest (Fig. 4).

Discussion

Food availability and group density of Japanese macaques were quite different between younger naturally regenerated forests and older plantation forests. In plantation forests 19 yr after logging, fruit production was negligible. The forest was composed mostly of *Cryptomeria japonica*, which offers male pollen cones as food only during the winter (Hanya, 2004). In contrast, in naturally regenerated forests, fruit availability was higher than in either primary or plantation forests for at least 7–18 yr after logging. Fruit availability is important because macaques in this region prefer fruits over other foods (Hanya, 2004), fruit availability is the principal determinant of the altitudinal variation in macaque density on Yakushima (Hanya et al., 2004), fruit production is known to affect reproduction in female Japanese macaques (Suzuki et al., 1998), fruit is high-quality food because of its high energy and low fiber content (Iwamoto, 1982), and fruit is generally a limited resource, whereas other foods, leaves in particular, are often superabundant (Janson & Chapman, 1999). Variation in the density of Japanese macaques was consistent with variation in food availability, at least for the plantation forest; macaque density was lowest in old plantation forests, where food availability was lowest.

In plantation forests, the macaques' food trees were cleared during logging. Even if they appear after logging, they are often cut to enhance the growth of *Cryptomeria japonica*. Once *Cryptomeria japonica* trees become large enough, short trees cannot reproduce under their shade. Our data indicate that these undesirable changes for frugivores, such as Japanese macaques, take place within 19 yr.

In young naturally regenerated forests, availability of food, and of fruit in particular, is higher than in primary and old plantation forests. Large fruiting trees are usually killed by clear-cutting, but this was not the case for the main sources of fruit for the macaques in this forest, as they are small trees. In cases of natural regeneration, felled areas are not cleared, and some small trees survive. When tall trees are cut, small trees receive more sunlight, which enhances their reproduction (Guariguata & Saenz, 2002). However, *Cleyera japonica* did not fruit in logged forest. The minimum DBH at which *Cleyera japonica* reproduced was 10 cm. This suggests that small *Cleyera japonica* in logged forests cannot reproduce like other species, even when light conditions are enhanced. Considering the observed interspecies variation in responses to logging, we propose that the effect of logging on frugivores varies with the flora. When large trees are the main source of fruit foods for frugivorous primates, their density and fruit availability are reduced by logging (Felton et al., 2003). When small trees offer a considerable amount of fruit, food availability for frugivores can be increased by logging (this study; Ganzhorn, 1995).

In conclusion, conversion of natural broad-leaved forest into monocultural *Cryptomeria japonica* forest is detrimental to the conservation of Japanese macaques. When small trees provide fruit for macaques, natural regeneration offers habitats of high fruit availability in the initial stage of regeneration, which lasts at least 7–19 yr after logging, and macaque density there is as high as in the primary forest.

References

Agetsuma N. (1998) Crop damage by wild Japanese monkeys on Yakushima Island, Japan. *Japanese Journal of Conservation Ecology* **3**: 43–55.

Felton A.M., Engstrom L.M., Felton A. & Knott C.D. (2003) Orangutan population density, forest structure and fruit availability in hand-logged and unlogged swamp forests in West Kalimantan, Indonesia. *Biological Conservation* **114**: 91–101.

Ganzhorn J.U. (1995) Low-level forest disturbance effects on primary production, leaf chemistry, and lemur populations. *Ecology* **76**: 2084–2096.

Guariguata M.R. & Saenz G.P. (2002) Post-logging acorn production and oak regeneration in a tropical montane forest, Costa Rica. *Forest Ecology and Management* **167**: 285–293.

Hanya G. (2004) Diet of a Japanese macaque troop in the coniferous forest of Yakushima. *International Journal of Primatology* **25**: 55–71.

Hanya G., Yoshihiro S., Zamma K., Kubo R. & Takahata Y. (2003) New method to census primate groups: estimating group density of Japanese macaques by point census. *American Journal of Primatology* **60**: 43–56.

Hanya G., Yoshihiro S., Zamma K., Matsubara H., Ohtake M., Kubo R., Noma N., Agetsuma N. & Takahata Y. (2004) Environmental determinants of the altitudinal variations in relative group densities of the Japanese macaques on Yakushima. *Ecological Research* **19**: 485–493.

Iwamoto T. (1982) Food and nutritional condition of free ranging Japanese monkeys on Koshima Islet during winter. *Primates* **23**: 153–170.

Janson C.H. & Chapman C.A. (1999) Resources and primate community structure. In: *Primate Communities* (eds J.G. Fleagle, C.H. Janson & K.E . Reed), pp. 237–267. Cambridge University Press, Cambridge.

Suzuki S., Noma N. & Izawa K. (1998) Inter-annual variation of reproductive parameters and fruit availability in two populations of Japanese macaques. *Primates* **39**: 313–324.

Takatsuki S. (1996) Conservation of common species. In: *Conservation Biology* (ed H. Higuchi), pp. 191–220. University of Tokyo Press, Tokyo.

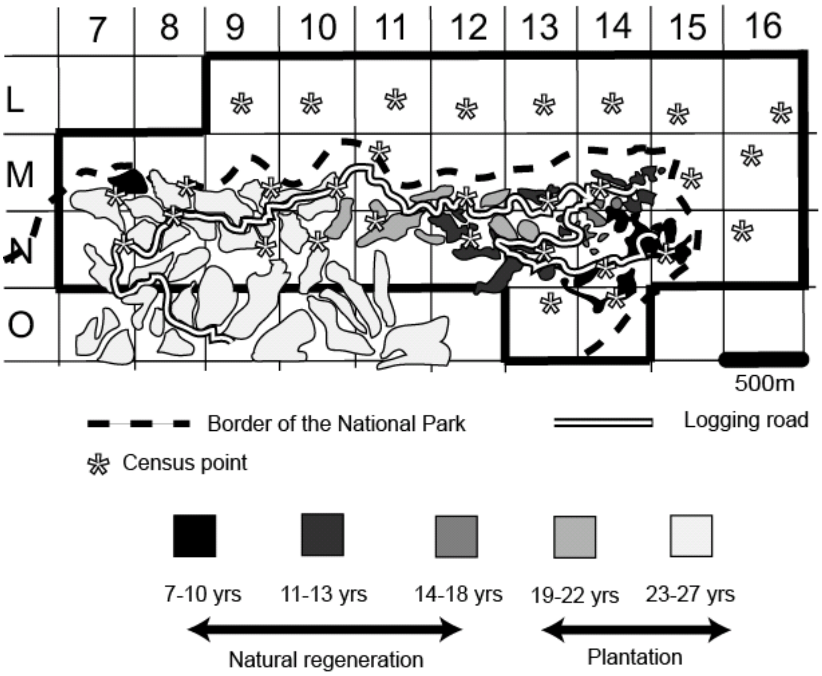


Fig. 1. Vegetation and the distribution of points where macaque groups were censused. Thick lines indicate the census area.

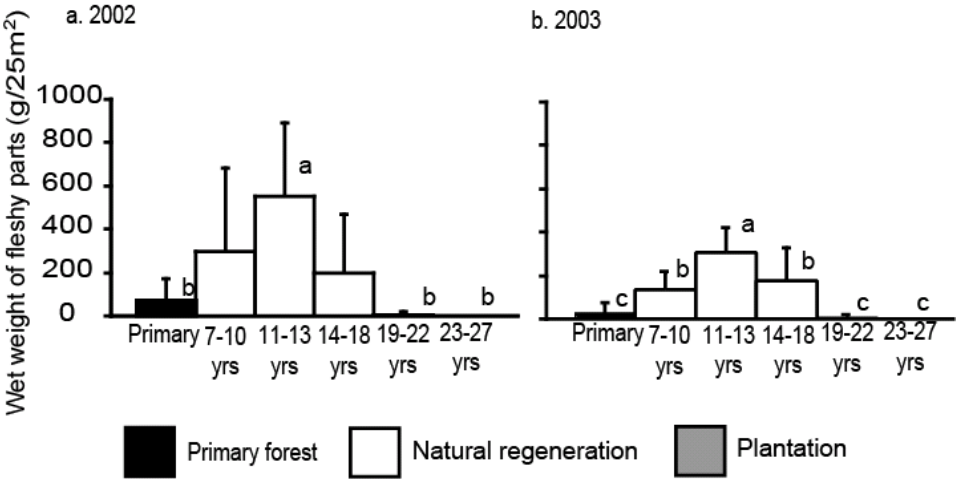


Fig. 2. Total annual fruit production. Means and standard deviations are shown.
Letters above the bars indicate significant differences between bars with different characters (in the order a > b > c).

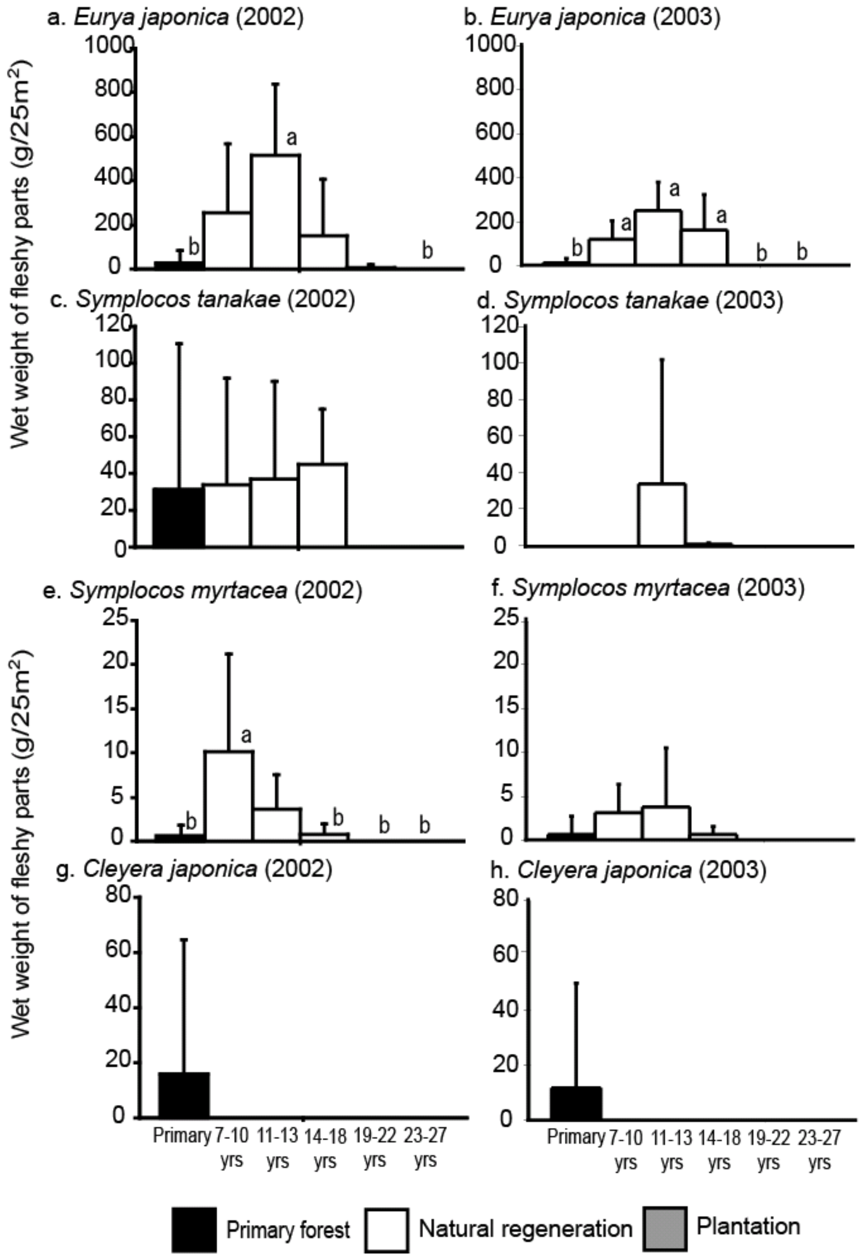


Fig. 3. Fruit production of the main species. Means and standard deviations are shown. Letters above the bars indicate significant differences between bars with different characters (in the order a > b > c).

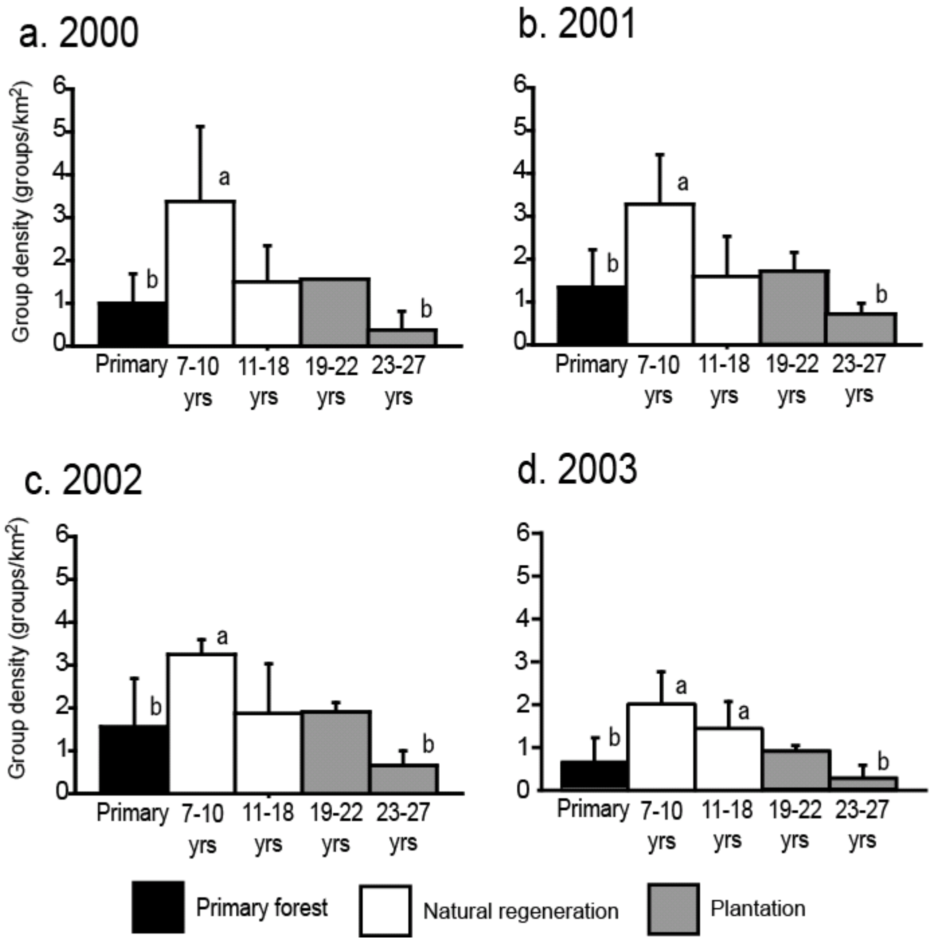


Fig. 4. Group density of Japanese macaques. Means and standard deviations are shown. Letters above the bars indicate that there are significant differences between bars with different characters, in the order of a>b>c

Changes in Plant Diversity after Conversion from Secondary Broadleaf Forest to *Cryptomeria* Plantation Forest: Chronosequencial Changes in Forest Floor Plant Diversity

Hiroshi Tanaka[1], Tetsuya Igarashi[2], Kaoru Niiyama[1], Mitsue Shibata[3],
Asako Miyamoto[1], Takuo Nagaike[4]
[1]Forestry and Forest Products Research Institute
[2] Kansai Research Center, Forestry and Forest Research Institute
[3] Tohoku Research Center, Forestry and Forest Research Institute
[4]Yamanashi Forest Research Institute

Introduction

The conservation of forest biological diversity is considered one of the most important criteria for ecologically sustainable forest management (Hunter 1999, Lindenmayer and Franklin 2002) because biological diversity is thought to be related to many ecological functions (Scherer-Lorenzen et al. 2005). This idea is still an open question. In addition to studies that focus on the relationships between biological diversity and ecological function, further information is required on the actual biological diversity of forested landscapes.

Conversions from primary broadleaf forests, coppice broadleaf forests, and semi-natural grasslands to conifer (sugi, *Cryptomeria japonica*, and hinoki, *Chamaecyparis obtusa*) plantations occurred after the 1940s in the mountainous areas of Japan, resulting in the replacement of a large portion of the natural and semi-natural vegetation of Japan by conifer plantations prior to the 1980s (Inoue 2003). Biological diversity sustained by traditional land use may have been negatively affected by the conversion of mixed stands to pure monocultures. Several studies have described the effects of such conversions on plant diversity in different regions and plantation forest types at the stand level (Nagaike 2002, Ito et al. 2003, 2004, Nagaike et al. 2005). However, few studies have investigated the dynamic chronosequential changes in plant diversity in both natural secondary forests and converted plantation forests (Nagaike 2003, Nagaike et al. 2006).

To sustainably manage a mosaic forest landscape composed of secondary broadleaf forests and conifer plantations, it is important to understand the plant diversity and how it changes with stand age of both forest types, as well as the contribution of each forest type to the maintenance of regional biodiversity. The appropriate zoning or spatial arrangement of stands for sustainable forest management depends on an understanding of the compositional characteristics of each forest type and age, as well as the dependence of plant species on forest type and age.

To obtain a scientific basis for plant diversity management and conservation, we addressed the following two specific questions. How does plant diversity change (recover) in both plantation and secondary natural forest stands after clear-cutting? How does conversion from deciduous broadleaf forest to evergreen conifer plantation affect species diversity? We used a chronosequence approach to compare sample stands of different ages scattered across a typical landscape.

Materials and methods

Study sites

The study was conducted in two areas located about 10 km apart: Ogawa and Satomi, Ibaraki Prefecture, central Japan. Both areas are located at the southern edge of the Abukuma Mountains (approximately 36º 50–56′ N, 140º 34–35′ E; 580–800 m above sea level). The annual mean temperature and precipitation in Ogawa are 10.7ºC and 1910 mm, respectively (Mizoguchi et al. 2002). The landscape of both areas is now mainly composed of deciduous broadleaf forests and plantations of *Cryptomeria japonica* (partly of *Chamaecyparis obtusa*), although the proportion of plantations is much higher in Satomi than in Ogawa (94% vs. 47% of the total forested area, respectively). In old-growth broadleaf forests, which are preserved only in Ogawa, the dominant canopy trees are *Quercus serrata*, *Fagus japonica*, and *F. crenata* (Masaki et al. 1992, Tanaka et al. in press). A large portion of the broadleaf forests in Ogawa, including the old-growth forests, has long been subjected to human activities such as burning, cattle grazing, and clear-cutting for fuel (Suzuki 2002). Small-scale (1–2 ha) clear-cutting with short intervals of ca. 30 years has been conducted repeatedly until now in the secondary broadleaf forests to make charcoal, produce pulp wood, and collect bed logs for mushroom culture. The conversion of pasture or secondary broadleaf stands to conifer plantations greatly increased the proportion of plantation forests in Japan after the Second World War (Inoue 2003). In addition, this land-use history resulted in a mosaic-like forest landscape in these areas, composed of secondary broadleaf stands and conifer plantations of various ages.

Field study

We examined the forest vegetation in 13 post-harvest secondary deciduous broadleaf forests (SB) and 26 conifer (*Cryptomeria japonica*) plantation forests (CP) along a chronosequence from just after clear-cutting to mature stands (70–80 years old). Four preserved old deciduous broadleaf forests (OG) were also studied as a reference for old-growth conditions. Plantation forests had been tended (i.e., weeded and thinned) according to the common schedule of the local governmental forest offices, but not so intensively managed as well-managed private plantations.

In each forest, we established a 10 × 100 m belt-shaped study plot at the center of each stand wherever possible to exclude the effects of neighboring stands of different management types. To minimize the influence of variation in site conditions, we selected stands located on gentle slopes, and the belt-shaped plots were designed to encompass the topographic variation in each stand (Iida and Nakashizuka 1995, Fukamachi et al. 1996, Nagaike 2002, Nagaike et al. 2003, 2006).

Each study plot was divided into 5 × 5 m quadrats, and a 1 × 1 m subquadrat was positioned in the corner of each quadrat. In total, we investigated 1060 quadrats and subquadrats:

160 in old-growth forests, 380 in secondary forests, and 520 in plantation forests. In each study plot, trees and woody vines > 2 m in height and with a diameter at breast height (dbh) > 5 cm were tagged, and their girth at breast height (gbh) was measured. Forest floor vegetation, i.e., < 2 m in height, was censused within each subquadrat using the Braun-Blanquet method. Standing dead stems > 5 cm in dbh were tagged and measured. We also recorded all woody plants > 2 m in height in each quadrat. Light conditions at the forest floor were measured in each subquadrat using hemispherical photographs taken 1 m above the ground.

A 200 × 300 m plot was established in the Ogawa Forest Reserve, and 2 × 2 m quadrats were placed at each intersection of a 10 × 10 m grid (651 quadrats in total) for the collection of additional data on forest floor vegetation in old-growth forests. The nomenclature follows Kitamura and Okamoto (1959), Kitamura et al. (1974, 1978), and Kitamura and Murata (1980).

Data analysis

To define understory vegetation species groups, the occurrence biases of species found in particular forests were tested using chi-square and Fisher's exact tests, based on the procedure of Nagaike et al. (2003). To include the vegetation data from the Ogawa Forest Reserve 2 × 2 m quadrats, we analyzed the pooled data from four 1 × 1 m subquadrats. In total, we investigated 1059 2 × 2 m subquadrats (691 in old-growth forests, 122 in secondary forests, and 246 in plantation forests) from four old-growth forests, 13 secondary broadleaf forests, and 26 plantation forests. We examined whether species occurred disproportionately in old-growth forests, secondary forests, or plantations. Species with no significant bias were classified as generalists, whereas those with occurrence frequencies ≤ 5 were classified as infrequent species. Thus, species were classified into five types according to their occurrence bias toward particular forest types. In addition, we classified the species listed in Numata and Yoshizawa (2002) as weed species, which are characterized as ruderal or disturbance-tolerant species (Bhuju and Ohsawa 1999, Kitazawa and Ohsawa 2002) and are usually considered undesirable from a management perspective. Species listed in the Red Data Book (Environment Agency of Japan 2000) did not occur in the study area.

To examine the differences between secondary forests and plantation forests in forest floor plant species abundance, analysis of variance (ANOVA) was performed for stands pooled into three age categories: young (3–10 years old), intermediate (10–40 years old), and mature (≥ 41 years old) stands (JMP, SAS Institute 2001). To test for compositional differences among forest types, cluster analysis and detrended correspondence analysis (DCA) were performed using PC-ORD (McCune and Mefford 1999). The species occurrence frequency in each plot was used for DCA. Correlations between the axis scores of DCA and stand structural and compositional variables reflecting management intensity were calculated using Kendall's τ (JMP, SAS Institute. 2001).

Results

Changes in stand structure and species diversity with stand age

As stand age increased, stand structural parameters such as basal area (BA) increased in both secondary forests and conifer plantations (Fig. 1). The BA was larger in plantation forests than in secondary forests (and old-growth forests) along the chronosequence (Fig. 1), although the maximum dbh was comparable in secondary forests and conifer plantations (data not shown).

Young stands (2–10 years after clear-cutting) had higher species richness of forest floor plant species (i.e., herbs and ferns) than did intermediate (11-40 years) and mature stands (>40 years) in both secondary and plantation forests (Tukey-Kramer’s HSD, $p < 0.01$; Fig. 2). The species richness of forest floor plant species was marginally higher in secondary forests than in plantation forests at each age category (ANOVA, $p = 0.05$; Fig. 2).

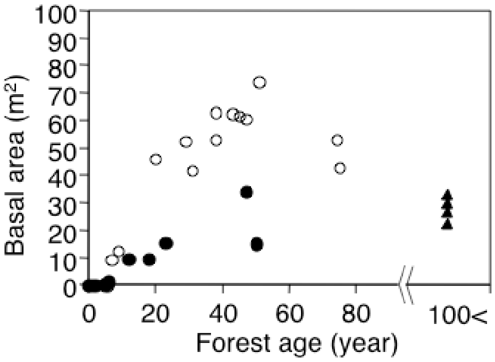


Fig. 1. Relationship between stand age and basal area (BA) of trees with diameter at breast height (dbh) > 5 cm. Open circle: conifer plantation; closed circle: secondary

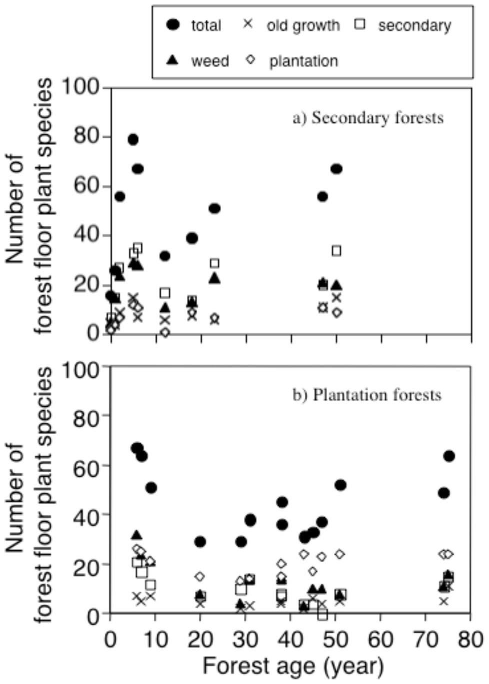


Fig. 2. Relationship between forest stand age and number of forest floor plant species in (a) secondary forests and (b) plantation forests.

Species diversity and composition in plantations and secondary forests

We recorded a total of 324 species (Fig. 3, Table 1). The three forest types appeared to contribute equally to the forest flora, although this result is based on restricted sampling (Fig. 3). According to the species occurrence biases, infrequent species (124 species, 38%) made up the highest percentage of species (Table 1). Secondary forest species (71 species, 22%) accounted for the second highest occurrence frequency, but plantation forest species accounted for nearly the same percentage (62 species, 19%). The secondary forest species group contained the highest percentage of weed species (41%). The old-growth forest species group contained the fewest weed species, but this was still a substantial percentage of the total species count (33 species, 10%).

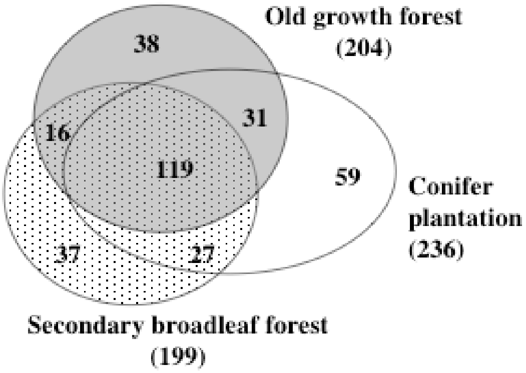


Fig. 3. Number of forest floor plant species in each forest type.

Table 1. Species groups classified with the distributional bias to the specific forest types.

Species group	No. of species	No. of weedy species (%)	Species
Old growth forest species	33	6 (18)	*Ainsliaea acerifolia* var. *subapoda*, *Pseudostellaria palibiniana*, *Salvia nipponica*, *Cynanchum magnificum*, *Viola eizanensis*, *Prenanthes acerifolia*
Secondary forest species	71	29 (41)	*Disporum smilacinum*, *Viola grypoceras*, *Hosta albo-marginata*, *Viola rossii*, *Luzula plumosa* var. *macrocarpa*, *Ixeris dentata*, *Carex lanceolata*, *Potentilla freyniana*
Plantation forest species	62	20 (32)	*Athyrium yokoscense*, *Oplismenus undulatifolius*, *Deparia conilii*, *Chloranthus serratus*, *Impatiens textori*, *Laportea bulbifera*, *Disporum sessile*, *Arachniodes borealis*
Others	158	39 (25)	
Common	34	7 (21)	*Carex sachalinensis* var. *alterniflora*, *Tricyrtis affinis*, *Elatostema umbellatum* var. *majus*, *Carex dolichostachya* var. *glaberrima*, *Arisaema serratum*, *Smilacina japonica*, *Cacalia farfaraefolia*, *Stellaria sessiliflora*
Infrequent species	124	32 (26)	*Caulophyllum robustum*, *Tulotis ussuriensis*, *Goodyera schlechtendaliana*, *Allium monanthum*, *Polygonatum involucratum*, *Chelidonium majus* var. *asiaticum*, *Scrophularia kakudensis*
Total	324	94 (29)	

Broadleaf (secondary and old-growth) and plantation forests were clearly distinguished by cluster analysis according to the forest floor plant species composition, although the difference between secondary forests and old-growth forests was not very distinct (Fig. 4). Broadleaf forests (secondary and old-growth forests) were also distinguished from plantation forests along axis 1 of the DCA ordination (Fig. 5). In addition, old-growth forests were distinguished from secondary forests along axis 2 (Fig. 5). According to the correlations between the DCA axis scores and forest age (Table 2), axis 2 was significantly positively correlated with forest age for both confer plantations and secondary forests. This is consistent with the placement of old-growth forests above secondary forests in the DCA ordination diagram (Fig. 2). For the plantation forests, the axis 1 scores were positively correlated with the proportion of plantation forest species and negatively correlated with the proportion of secondary forest species (Table 2). The axis 1 scores for secondary forests were negatively correlated with the proportion of secondary forest species (Table 2). The proportion of weed species was negatively correlated with axis 1 for secondary forests and axis 2 for plantation forests (Kendall's rank correlation, $p < 0.05$, Table 2).

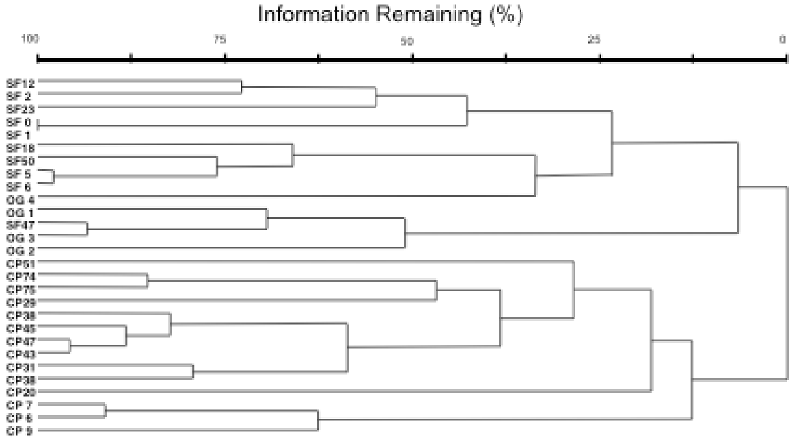


Fig. 4. Dendrogram showing the result of cluster analysis. Number in stand name indicates stand age.

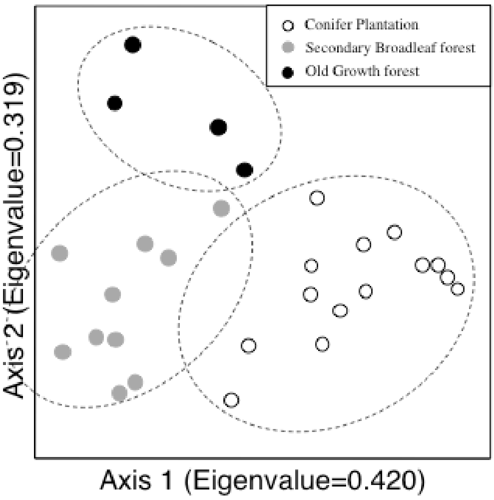


Fig. 5. Diagram of the results of detrended correspondence analysis (DCA). Each symbol indicates a stand.

Table 2. Correlation between the two axes of DCA and the forest age and the proportion of each species group (Kendall's rank correlation coefficient).

	Forest age	Proportion of old growth forest species	Proportion of secondary forest species	Proportion of plantation forest species	Proportion of weed species
Conifer plantation					
Axis 1	0.287	-0.022	-0.539**	0.473*	-0.275
Axis 2	0.729**	0.155	-0.407*	0.385†	-0.495*
Secondary broadleaf forest					
Axis 1	0.500†	0.389	-0.611*	0.222	-0.556*
Axis 2	0.556*	0.556*	-0.444	0.278	-0.389

*: p < 0.05, **: p < 0.01. †: marginally significant.

Ecological attributes of species in plantations and secondary forests

The proportion of weedy species was negatively correlated with stand age in both plantation and secondary forests (τb = –0.500, p = 0.061 for secondary forests; τb = –0.464, p = 0.021 for plantation forests; Fig. 6). The number of weedy species was highest approximately 5 years after clear-cutting in both secondary forests and plantation forests (Fig. 2). The proportion of secondary forest species was negatively correlated with stand age in plantation forests (τb = –0.420, p = 0.037; Fig. 6). The proportion of plantation forest species showed no significant correlation with stand age in either of the forest types (Fig. 6). The proportion of old-growth forest species showed a marginally positive correlation with stand age in secondary forests (τb = 0.444, p = 0.095; Fig. 6).

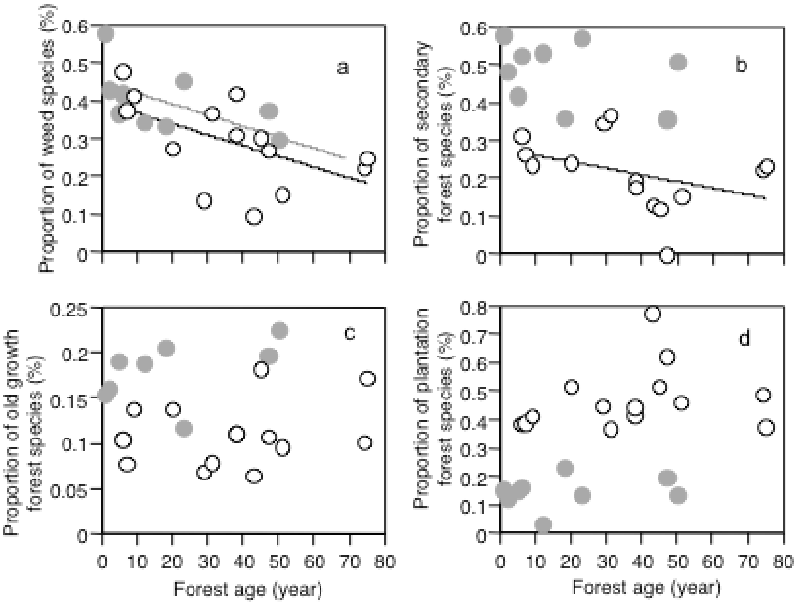


Fig. 6. Relationship between forest stand age and the proportion of four species types: (a) weed species, (b) secondary forest species, (c) old-growth forest species, and (d) plantation forest species.

Discussion

Although species richness differed only marginally between secondary and plantation forests along a chronosequence after clear-cutting and showed similar patterns of change along the chronosequence (Fig. 2), the conversion from secondary forests to plantation forests resulted in a distinctly different forest floor plant species composition (Figs. 4, 5). This difference is understandable because the conversion from deciduous broadleaf forest to evergreen conifer forest results in different microenvironments according to changes in canopy leaf composition and phenology (Ramovs and Roberts 2003). After canopy closure (ca. 10 years after clear-cutting), seasonal light, temperature, and moisture conditions are likely to be more temporally stable and spatially homogeneous in plantation forests than in secondary forests. Larger stem basal area in plantation forests relative to secondary forests (Fig. 1) may also contribute to the differences in forest floor microenvironments.

In both secondary forests and plantation forests, weeds accounted for a high percentage of species, suggesting the influence of severe disturbance; i.e., clear-cutting, in both forest types. The rather high percentage of weed species also found in old-growth forests may reflect the influence of large-scale disturbances, e.g., fire and grazing by horses and cattle, that frequently occurred in the past in this region (Suzuki 2002, Miyamoto and Sano 2007). The proportion of weed species decreased with stand age (time since disturbance) in both secondary and plantation forests (Fig. 6, Table 2). Colonization by a large number of weed species partly explained the high species diversity after clear-cutting (Fig. 2; Fahy and Gormally 1998, Bhuju and Ohsawa 1999, Nagaike 2000). Higher species richness in intensely managed stands has also been reported in many types of managed forest (Schoonmaker and McKee 1988, Brunet et al. 1996, Stapanian et al. 1998, Scherer et al. 2000).

Although the number and proportion of weed species decreased with stand age in both secondary and plantation forests, the results of DCA ordination and the relationships between the DCA axes and forest age suggest that the compositions of these forest types were not converging as stand age increased (Fig. 5). The proportion of secondary forest species in plantation forests decreased with stand age (Fig. 6). In contrast, the proportion of old-growth forest species did not change as stand age increased, even in secondary forests, suggesting that in the first 60 years post-clear-cutting in secondary that the abandonment of secondary coppice forests does not directly lead to the recovery of old-growth forest plant species (Ramovs and Roberts 2003). Thus, the preservation of the remaining old-growth forests is very important for the conservation of regional plant species diversity.

Relative to old-growth forests, both secondary forests and conifer plantations contributed substantially to local plant species diversity, although both contained large percentages of weed species (Fig. 3, Table 1). The plantation species included an abundance of ferns that may have taken advantage of the preferable environmental conditions of plantation forests (Table 1). Among the infrequent species, orchids were also found in plantation forests (Table 1). In contrast, most of the spring ephemerals such as *Erythronium japonicum* and *Anemone pseudo-altaica* were only found in deciduous broadleaf forests (secondary and old-growth forests). Plantation forests may contribute to local plant diversity, but cannot substitute for secondary and old-growth forests (Maeto et al. 2002, Inoue 2003).

To quantitatively evaluate the contribution of conifer plantations and secondary forests of different ages to the maintenance of local plant diversity, further understanding of the processes and mechanisms shaping species composition is necessary (Halpern 1989, Roberts and Gilliam 1995). In addition to the occurrence bias of forest floor plant species, information on the functional types (e.g., growth form, dispersal type, pollination type; Smith et al. 1997) of the component species will be important in understanding these processes and mechanisms.

References

Brunet J, Falkengren-Grerup U, Tyler G (1996) Herb layer vegetation of south Swedish beech and oak forests — effects of management and soil acidity during one decade. For Ecol Manage 88: 259–272

Bhuju D R, Ohsawa M (1999) Species dynamics and colonization patterns in an abandoned forest in an urban landscape. Ecol Res 14: 139–153

Environmental Agency of Japan (2000) Threatened wildlife of Japan—Red Data Book. Vascular plants. 2nd ed. Japan Wildlife Research Center, Tokyo.

Fahy O, Gormally M (1998) A comparison of plant and carabid beetle communities in an Irish oak woodland with a nearby conifer plantation and clearfelled site. For Ecol Manage 110: 263–273

Fukamachi K, Iida S, Nakashizuka T (1996) Landscape patterns and plant species diversity of forest reserves in the Kanto region, Japan. Pl Ecol 124: 107–114

Halpern C B (1989) Early successional patterns of forest species: interactions of life history traits and disturbance. Ecology 70: 704–720

Hunter Jr, M L ed (1999) Maintaining biodiversity in forest ecosystems. Cambridge University Press, Cambridge

Iida S, Nakashizuka T (1995) Forest fragmentation and its effect on species diversity in sub-urban coppice forests in Japan. For Ecol Manage 73: 197–210

Inoue T (2003) Chronosequential change in a butterfly community after clear-cutting of deciduous forests in a cool temperate region of central Japan. Entomol. Sci. 6: 151–163

Ito S, Nakagawa M, Buckley G P, Nogami K (2003) Species richness in sugi (Cryptomeria japonica D. Don) plantations in southeastern Kyushu, Japan: The effects of stand type and age on understory trees and shrubs. J For Res 8: 49–57

Ito S, Nakayama R, Buckley G P (2004) Effects of previous land-use on plant species diversity in semi-natural and plantation forests in a warm-temperate region in southeastern Kyushu, Japan. For Ecol Manage196: 213–225

Kitamura S, Murata G (1980) Colored illustrations of herbaceous plants of Japan (Choripetalae). Hoikusya Publishing Co. Ltd., Osaka, Japan. [In Japanese.]

Kitamura S, Murata G, Hori M (1978) Colored illustrations of herbaceous plants of Japan (Sympetalae).

Hoikusya Publishing Co. Ltd., Osaka, Japan. [In Japanese.]

Kitamura S, Murata G, Koyama T (1974) Colored illustrations of herbaceous plants of Japan (Monocotyledoneae). Hoikusya Publishing Co. Ltd., Osaka, Japan. [In Japanese.]

Kitamura S, Okamoto S (1959) Colored illustrations of trees and shrubs of Japan. Hoikusya Publishing Co. Ltd., Osaka, Japan. [In Japanese.]

Kitazawa T, Ohsawa M (2002) Patterns of species diversity in rural herbaceous communities under different management regimes, Chiba, central Japan. Biol Conserv 104: 239–249

Lindenmayer D B, Franklin J F (2002) Conserving forest biodiversity: a comprehensive multiscaled approach. Island Press, Washington, D.C.

Maeto K, Sato S, Miyata H (2002) Species diversity of longicorn beetles in humid warm-temperate forests: the impact of forest management practices on old-growth forest species in southwestern Japan. Biodiv Conserv 11: 1919–1937.

Masaki T, Suzuki W, Niiyama K, Iida S, Tanaka H, Nakashizuka T (1992) Community structure of a species rich temperate forest, Ogawa Forest Reserve, central Japan. Vegetatio 98: 97–111

McCune B, Mefford M J (1999) PC-ORD: multivariate analysis of ecological data; version 4 [computer program]. MjM Software Design, Gleneden Beach, Oregon.

Miyamoto A, Sano M (2007) The influence of change in forest resource use on forest landscapes in Abukuma mountainous area in the past 90 years. this volume.

Mizoguchi Y, Morisawa T, Ohtani Y (2002) Climate in Ogawa Forest Reserve. Ecol studies 158: 11–18.

Nagaike T (2002) Differences in plant species diversity between conifer (Larix kaempferi) plantations and broad-leaved (*Quercus crispula*) secondary forests in central Japan. For Ecol Manage 168 (1): 111–123

Nagaike T, Hayashi A, Abe M, Arai N (2003) Differences in plant species diversity in *Larix kaempferi* plantations of different ages in central Japan. For Ecol Manage 183: 177–193

Nagaike T, Kamitani T, Nakashizuka T (2005) Effects of different forest management systems on plant species diversity in a *Fagus crenata* forested landscape of central Japan. Can J For Res 35: 2832–2841

Nagaike T, Hayashi A, Kubo M, Abe M, Arai N (2006) Plant species diversity in a managed forest landscape composed of *Larix kaempferi* plantations and abandoned coppice forests in central Japan. For Sci 52 (3): 324–332

Numata M, Yoshizawa N eds (2002) Weed flora of Japan. Zenkoku Noson Kyoiku Kyoukai, Tokyo. [In Japanese.]

Ramovs B V, Roberts M R (2003) Understory vegetation and environment responses to tillage, forest harvesting and conifer plantation management. Ecol Appl 13: 1682–1700

Roberts M R, Gilliam F S (1995) Patterns and mechanisms of plant diversity in forested ecosystems: implications for forest management. Ecol Appl 5: 969–977

SAS Institute (2001) JMP user’s guide. Version 5.0.1J, SAS Institute, Cary, North Carolina, USA.

Scherer G, Zabowski D, Java B, Everett R (2000) Timber harvesting residue treatment. Part II. Understory vegetation response. For Ecol Manage 126: 35–50

Scherer-Lorenzen M, Körner Ch, E.-D Schulze eds (2005) Forest Diversity and Function: Temperate and Boreal Systems, Springer Verlag, Berlin.

Schoonmaker P, McKee A (1988) Species composition and diversity during secondary succession of coniferous forests in the western Cascade Mountains of Oregon. For Sci 34: 960–979

Smith T M , Shugart H H & Woodward F I eds (1997) Plant functional types: their relevance to ecosystem properties and global change. Cambridge University Press, Cambridge.

Stapanian M A, Sundberg S D, Baumgardner G A, Liston A (1998) Alien plant species composition and associations with anthropogenic disturbance in North American forests. Pl Ecol 139: 49–62

Suzuki W (2002) Forest vegetation in and around Ogawa Forest Reserve in relation to human impact. Ecol Studies 158: 25–41

Tanaka H, Shibata M, Masaki T, Iida S, Niiyama K, Abe S, Kominami Y, Nakashizuka T (2007) Comparative demography of three coexisting maples in gaps and under a closed canopy. J Veg Sci, in press

Understory Herbaceous Species Composition Depends on Tree Canopy Dynamics in an Old-Growth Forest, Ogawa Forest Reserve, Northern Japan

Takuo Nagaike[1], Hiroshi Tanaka[2], Kaoru Niiyama[2], Mitsue Shibata[3], Tetsuya Igarashi[4], Masaaki Takyu[5]
[1]*Yamanashi Forest Research Institute*
[2]*Forestry and Forest Products Research Institute*
[3]*Tohoku Research Center, Forestry and Forest Products Research Institute*
[4]*Kansai Research Center, Forestry and Forest Products Research Institute*
[5]*Tokyo University of Agriculture*

Introduction

Forest biota are adapted to and maintained by natural disturbance within the forest (e.g., Attiwill 1994). Therefore, it is often considered that one option for ecologically sustainable forest management is to emulate the intensity, frequency, and period of natural disturbance (e.g., Seymour and Hunter 1999; Lindenmayer and Franklin 2002; Mitchell et al. 2002; Crow and Perera 2004). Studies of the effects of natural disturbance in maintaining species diversity have increased in frequency (e.g., Nakashizuka 2001). In particular, canopy gap creation, a major source of natural disturbance in forest canopies and the focal point for consequent natural regeneration of trees, has been studied in relation to gap size (e.g., Abe et al. 1995; Vandenberghe et al. 2006) and microsites after gap creation (e.g., Carlton and Bazzaz 1998). However, most studies have only examined the responses of canopy dynamics of tall tree species and few have applied the results to forest management (e.g., Emborg et al. 2000).

The major source of natural disturbance in Ogawa Forest Reserve in northern Japan is canopy gap creation (Nakashizuka 2002). Therefore, when considering forest management practices to match the intensity, frequency, and period of canopy dynamics in the region, one should consider the understory species involved in the canopy dynamics. Species classification according to canopy dynamics has mainly focused on shade tolerance (e.g., Abe et al. 1995). However, to emulate natural disturbance in a target region forest management should focus on several species traits, not only shade-tolerance but also disturbance tolerance, because logging always involves physical disturbance to forest dynamics. Few studies have focused on species with tolerance to disturbance. Hence, in this study we evaluated species canopy dynamics in an old-growth forest in the Ogawa Forest Reserve with special reference to specific habitat types of species in the landscape.

Study site and methods

Study site

This study was conducted in the Ogawa Forest Reserve and the surrounding forested landscape, in Ibaraki Prefecture, northern Japan. The landscape consists mainly of deciduous broadleaf old-growth forests, coppice forests, and coniferous plantations. We conducted vegetation surveys in these three major forest types. For the deciduous broadleaf old-growth forest (hereafter, “old-growth”), we studied the Ogawa Forest Reserve and the reserved belt that maintains features of an old-growth forest (e.g., well-developed stand structure and large maximum stem size). In the coppice forest (hereafter, “coppice forest’), some stands were actively managed for fuel and firewood, but some, in particular stands 30 or more years old, had been abandoned. *Cryptomeria japonica*, an evergreen conifer and important commercial timber species in Japan, is planted in the coniferous plantations. The plantation stands sampled for this study (hereafter, “plantation”) had a wide age range and management intensity, indicating the diverse management aims and motivations of forest owners or managers. For example, weeding and thinning were carried out on schedule in some stands, while other stands were almost abandoned, without any management occurring after planting.

Plant survey

A plot (200 × 300 m) was established and divided into a 10 × 10-m grid in the Ogawa Forest Reserve. A 2 × 2-m quadrat was set up at the intersection of each grid (651 quadrats in total). Beside the Ogawa Forest Reserve, 43 stands (old-growth: 4; coppice forest: 13; and plantation: 26) were examined. A belt 10-m wide and 100-m long was established in each stand. To exclude the effects of different management types in neighboring stands, study plots were set up at the center of each stand, where possible. The belt-shaped plots were designed to cover topographic variations in each stand and thus minimize variation in natural conditions (e.g., slope aspect and soil type) among the stands (Iida & Nakashizuka 1995; Fukamachi et al. 1996; Nagaike et al. 2005). Basically, each coppice forest and plantation, except for those less than 10 years old, had not been managed for at least 5 years. Coppice forest and plantation stand age ranged from 1 to 77 and from 4 to 76 years, respectively. Each belt-shaped plot was divided into 5 × 5-m grids; a 1 × 1-m quadrat was placed at the corner of every 5 × 5-m grid square. To analyze the different quadrat sizes (i.e., 2 × 2 m in Ogawa Forest Reserve and 1 × 1 m in belt-shaped plots), we transformed the data of four 1 × 1 m plots into 2 × 2 m in the belt-shaped plots.

The total number of quadrats investigated was 1059 (old-growth: 691; coppice forest: 122; and plantation: 246). All herbaceous species, including ferns, less than 2-m tall occurring in each 1 × 1-m quadrat were recorded. The survey was carried out twice (1991 and 2001) in the Reserve and once from 2001 to 2004 in the other stands.

Canopy dynamics

In the Ogawa Forest Reserve plot, canopy height above each quadrat (for 651 quadrats) was measured with a measurement pole in 1989 and 2001. We classified the canopy height in each survey into three categories: “gap”: $\leq$10 m; “development”: <10 m to $\leq$ 15 m; and “closed”: >15 m. The canopy dynamics are shown comparing the height of the canopy in 1989 and 2001.

Analysis

Frequency of species occurrence was summed for each forest type (old-growth, coppice forest, and plantation). To delineate the habitat types for each species, the occurrence biases of species found in particular habitats were analyzed using chi-square tests, based on procedures in Nagaike et al. (2003, 2005). All species were divided into three habitat groups, according to whether they occurred disproportionately in the old-growth forest (Old-growth forest species), coppice forest (Coppice forest species), plantation (Plantation species), or were without bias to a particular forest type or too infrequent for statistical analysis (Other species). We also examined whether a species is considered to be a weed (Numata & Yoshizawa 2002).

Results and discussion

We recorded a total of 353 species: 208 in old-growth forest, 206 in coppice forest, and 246 in the plantation. Of these 353 species, Other species (249 species, e.g., *Blechnum niponicum*, *Carex fernaldiana*, and *Smilax sieboldii*) made up the highest proportion, followed by Old-growth forest species (57 species, e.g., *Ainsliaea acerifolia* var. *subapoda*, *Pertya robusta*, and *Pseudostellaria palibiniana*). Coppice forest species and Plantation species accounted for 23 (e.g., *Carex lanceolata*, *Viola grypoceras*, and *Ixeridium dentatum*) and 24 (e.g., *Athyrium yokoscense*, *Deparia conilii*, and *Bidens frondosa*) species, respectively. The ratio of weed species to the number of species of each forest type was highest for Coppice forest (52%), followed by Plantation (38%), and Old-growth forest (16%).

In old-growth forest, “closed” canopy increased in frequency from 569 quadrats in 1989 to 596 quadrats in 2001. Consequently, both “development” and “gap” canopy types decreased in frequency (from 41 to 33 “development” quadrats and from 41 to 22 “gap” quadrats). In quadrats where “gap” changed to “closed” (i.e., gap closure) from 1989 to 2001, newly-occurring species were mainly Old-growth forest species (eight of 14 species). Lost species in the quadrats constituted 17 species, mostly Old-growth forest species (eight species). In quadrats where “closed” changed to “gap” (i.e., canopy gap creation), there were 14 newly-occurring species, of which eight were Old-growth forest species (Table 1). Four of the eight lost species were Old-growth forest species.

Fewer weed species, which were expected to be tolerant to severe disturbance, were found in old-growth forest, and more were found in coppice forest and plantations. This indicates that coppice forest and plantations suffered more severe disturbance than old-growth forest. Species that were new or that had disappeared from gap-created and canopy-closure quadrats were mainly Old-growth forest species. Therefore, we concluded that species adapted to old-growth forest canopy dynamics were native to old-growth forest, but differed from species which occurred after severe disturbance (i.e., Coppice forest and Plantation species). Consequently, we need to establish forest management procedures that maintain the diversity of Old-growth forest understory species.

References

Abe S, Masaki T, Nakashizuka T (1995) Factors influencing sapling composition in canopy gaps of a temperate deciduous forest. Vegetatio 120: 21-32.

Attiwill PM (1994) The disturbance of forest ecosystems: the ecological basis for conservative management. For Ecol Manage 63: 247-300.

Carlton GC, Bazzaz FA (1998) Regeneration of three sympatric birch species on experimental hurricane blowdown microsites. Ecol Monog 68: 99-120.

Crow TR, Perera AH (2004) Emulating natural landscape disturbance in forest management – an introduction. Landscape Ecol 19: 231-233.

Emborg J, Christensen M, Heilmann-Clausen J (2000) The structural dynamics of Suserup Skov, a near-natural temperate deciduous forest in Denmark. For Ecol Manage 126: 173-189.

Fukamachi K, Iida S, Nakashizuka T (1996) Landscape patterns and plant species diversity of forest reserves in the Kanto region, Japan. Vegetatio 124: 107-114.

Iida S, Nakashizuka T (1995) Forest fragmentation and its effect on species diversity in sub-urban coppice forests in Japan. For Ecol Manage 73: 197-210.

Mitchell RJ, Palik BJ, Hunter Jr. ML (2002) Natural disturbance as a guide to Silviculture. For Ecol Manage 155:

315-317.

Lindenmayer DB, Franklin JF (2002) Conserving forest biodiversity – A comprehensive multiscaled approach. Island Press, Washington.

Nakashizuka T (2001) Species coexistence in temperate, mixed deciduous forests. TREE 16: 205-210.

Nakashizuka T (2002) Disturbance regimes. In: Nakashizuka T, Matsumoto Y. (eds) Diversity and interaction in a temperate forest community. Springer, Tokyo.

Nagaike T, Kamitani T, Nakashizuka T (2003) Plant species diversity in abandoned coppice forests in a temperate deciduous forest area of central Japan. Plant Ecol 166: 63-74.

Nagaike T, Kamitani T, Nakashizuka T (2005) Effects of different forest management systems on plant species diversity in a Fagus crenata forested landscape of central Japan. Can J For Res 35: 2832-2840.

Numata M, Yoshizawa N. (eds.) (2002) Weed flora of Japan. Zenkoku Noson Kyoiku Kyoukai, Tokyo (in Japanese).

Seymour RS, Hunter Jr. ML (1999) Principles of ecological forestry. In: Hunter Jr. ML (ed) Maintaining biodiversiry in forest ecosystems. Cambridge University Press, Cambridge.

Vandenberghe C, Frelechoux F, Gadallah F, Buttler A (2006) Competitive effects of herbaceous vegetation on tree seedling emergence, growth and survival: does gap size matter? J Veg Sci 17: 481-488.

Table 1. The responses of plant species to changes in canopy dynamics in the Ogawa Forest Reserve

	From "gap" to close"		From "close" to "gap"	
Species types	Newly occurred species	Disappeared species	Newly occurred species	Disappeared species
Old-growth forest species	8	8	8	4
Coppice forest species	1		1	1
Plantation species		3	3	
Others	5	6	3	3
Total	14	17	14	8

Effects of Forestry Activities on Insect Biodiversity in Abukuma, Kanto Region, Temperate Japan

Shun'ichi Makino, Hideaki Goto, Kimiko Okabe, Takenari Inoue, Motohiro Hasegawa, Masahiro Sueyoshi, Keiko Hamaguchi, Hiroshi Tanaka, Isamu Okochi
Division of Forest Entomology, Forestry and Forest Products Research Institute
Matsunosato 1, Tsukuba, Ibaraki 305-8687 Japan

Introduction

Forestry activities affect animal biodiversity in various ways and degrees by modifying forests and their surroundings that are inhabited by such organisms. As animals perform various functions in the forest ecosystem, forestry practices should be selected so as to retain, or at least not produce severe deterioration of, their biodiversity. However, it is often unclear how different animal groups respond to particular forestry practices in terms of their biodiversity.

In the Abukuma area, northern Kanto, temperate Japan, there have typically been two types of forestry practice: 1) small scale clear-cutting of deciduous broad-leaved trees, and 2) conversion of broad-leaved stands into coniferous plantations of Japanese cedar (*Cryptomeria japonica*) and hinoki cypress (*Chamaecyparis obtusa*). In order to learn how these practices have affected animal biodiversity, we investigated species richness and assemblage of insects in differently managed stands. Because insects not only have the greatest biodiversity among animals (e.g. Grimaldi and Engel 2005), but perform a wide range of ecosystem functions (Weisser and Siemann 2004), we consider them to be some of the best target animals for evaluation of effects of forestry activities on biodiversity.

In this report, we first show how species richness of insects changes with the age of regeneration stands after clear-cutting of broad-leaved trees, with special reference to differential responses among insect taxa (cf. Makino et al. 2006). Besides insects, some mites (Acari) were also monitored. Secondly, we compare insect assemblage as well as species richness between broad-leaved and conifer stands of various ages, focusing on longhorn beetles (Coleoptera, Cerambycidae) (cf. Makino et al. 2007). Longhorn beetles are useful indicators of forest conditions because they are mainly xylophagous in larval stages and frequently visit flowers as adults, thus are expected to be sensitive to changes in forest conditions. Finally, we briefly discuss effects of forestry on insect biodiversity in this region.

Materials & Methods

Study areas

This study was conducted in 2 areas, Ogawa and Satomi, Ibaraki Prefecture, central Japan. Both areas had plantations of *Cryptomeria japonica* and *Chamaecyparis obtusa*, though the percentage of plantation areas was much larger in Satomi than in Ogawa (94% vs. 47% of total forested area). Ogawa is located at the southern edge of the Abukuma Mountains in Kitaibaraki (approximately 36° 56' N, 140° 35' E, 580–800 m a.s.l.). In deciduous broad-leaved forests in the area, the dominant large trees are *Quercus serrata*, *Quercus mongolica* and *Fagus crenata*. Broad-leaved stands have repeatedly been clear-cut on a small scale to collect bed logs for mushroom culture, and this continues in the present. In addition, the number of conifer plantations converted from broad-leaved stands greatly increased after the Second World War. These practices have resulted in a mosaic-like landscape composed of secondary broad-leaved stands and conifer plantations of different ages. We selected 10 plots in broad-leaved stands to form a chronosequence from 1 to over 100 years after clear-cutting (Table 1). All plots were located within an approximately 30 km^2 area. Satomi (approximately 36° 50' N, 140° 34' E, 700-800 m a.s.l.) is about 10 km southwest of Ogawa area. We selected 8 plots of Japanese cedar plantations in an approximately 10 km^2 area in Satomi. The conifer plots also formed a chronosequence from 1 to 76 years after plantation.

Table 1. Study plots and the number of longicorn species collected in Ogawa (broad-leaved plots) and Satomi (conifer plots) (modified from Makino et al. 2007).

Area	Plot code	Age (year)	Area (ha)
Ogawa	O1	1	3
	O4	4	5
	O12	12	4
	O24	24	24
	O51	51	10
	O54	54	14
	O71	71	19
	O128	>100	98
	O174	>100	11
	O178	>100	10
Satomi	S3	3	4
	S7	7	6
	S9	9	5
	S20	20	5
	S29	29	14
	S31	31	12
	S75	75	3
	S76	76	3

Monitoring methods and target animals

Target animals were selected from a variety of taxa representing different ecological functions. All insects and mites were collected in the 10 broad-leaved plots in Ogawa in 2002; for longicorn beetles, additional collection was made in the 8 conifer plots in Satomi in 2003. Targets and collection methods are as follows. Insect traps, when used, were placed well inside the study plots to avoid possible edge effects.

Butterflies: One-hour transect counts were made twice a month from April to October between 9:00 and 15:00 (cf. Inoue, 2003). Species and number of butterflies sighted were recorded at each census. **Moths**: A single portable light trap (Okochi, 2002) was left overnight at each of the monitoring sites twice in August, and moths trapped inside were collected the following morning. All plots were censused on the same nights. **Ground beetles**: Pitfall traps were used, made from transparent plastic bottles (77 mm diameter, 158 mm height) with three small holes (about 5 mm in diameter) in the middle for drainage of rainwater. In each plot, ten pitfall traps were aligned in three parallel lines, with a distance of about 10 m between traps. Trapped insects were collected every two weeks from April to November. **Tube-renting bees and wasps**: Nesting traps, which were made of 16 bamboo stalks (approximately 8 to 16 mm in diameter) and four reeds (6 mm), were used. At each plot, nine traps were tied to tree trunks or wooden posts (where no substrate trees are available) at 1.5 m above the ground in April, and removed in November. Immatures found in the traps were reared until eclosion for identification. **Longicorn beetles, hoverflies and fruit flies**: Collection was made with Malaise traps (Golden Owl Publishers, 180 cm long, 120 cm wide, 200 cm high). Five traps were set in April at each plot at intervals of 10 m. Collection of trapped insects was made every two weeks as in pitfall traps. **Ants**: Litter sampling was made between July and August along a 100 m (or 200 m in a few sites) transect line in each plot, and ants were hand-sorted. Additionally, pitfall traps (disposable plastic cups) were set along the same line at 10 m intervals for a maximum duration of three days. **Oribatid mites and collembola**: A cylindrical core (25 cm^2 x 5 cm high) of soil was removed from eight divisions (4 x 2 m) of a quadrat (8 x 8 m) in April, August, and November; soil arthropods were later extracted with Tullgren funnels (cf. Hasegawa et al., 2004). **Mites associated with mushrooms**: Mushroom fruiting bodies were sampled in each plot once a month from April to November. Mites were hand-sorted from mushrooms and identified under a microscope.

Forest characteristics

In order to learn how forest characteristics affect insect biodiversity, we made plant censuses in the above plots. A 100 m line transect was established at each plot from September 2000 to October 2003. All trees and vines of at least 2 m in height and at least 5 cm in DBH were counted and their GBH (girth at breast height) was measured in a total of forty 5 x 5 m quadrats along both sides of the 100 m transect line mentioned above; the frequency of trees smaller than 5 cm in DBH in the 40 quadrats was also censused. Forest floor vegetation with a height of less than 2 m (forest floor plants) was censused following the Braun-Blanquet method for a 1 x 1 m subquadrat in each 5 x 5 m quadrat.

Analyses of longicorn assemblages

Canonical Correspondence Analysis (CCA) of longicorn beetles was performed with Canoco for Windows, Version 4.5 (ter Braak and Smilauer 2002). Only those species with a total count of at least 3 individuals were used in CCA. As environmental variables, we selected the following plant community indices: species richness of plants for 3 size classes (trees with DBH $\geq$ 5 cm, trees with DBH <5 cm, and forest floor plants), the density of trees with DBH $\geq$ 5 cm and maximum and average diameter of tress at breast height. In this analysis, the scores of the 1st and 2nd axes in Detrended Correspondence Analysis (DCA) for the plant community group ordination for the 3 size classes were also used, in order to investigate the effects of plant species compositions on communities of longicorns. In DCA of plants, species with at least 3 individuals in total were used, and population data were transformed using logarithmic transformation, log10(x+1). In DCA of trees with DBH $\geq$ 5 cm, the 1-year-old site (O1) and the 4-years-old site (O4) were excluded because there were no trees of this size class. Likewise, in the DCA of forest floor plants, the 1-year-old site was excluded. Population data were transformed using logarithmic transformation, log10(x+1). Environmental variables were tested using forward selection of variables with Monte Carlo test using 499 unrestricted permutations ($P < 0.05$).

Results

Insect species richness in relation to the age of broad-leaved stands

Species richness of insects in broad-leaved stands in Ogawa is shown in Figure 1, in which the number of species is represented as a proportional rather than absolute value. The response of species richness to the stand age was classified into the following three types. Type I (butterflies, hoverflies, fruit flies, tube-renting bees and wasps, and longicorn beetles): species richness was high in grasslands or in early stages of succession, while much lower in older forests. Type II (mites associated with mushrooms): species richness was low in early stages of succession, becoming greater as forests mature. The number of mushroom species showed a similar response to the mite. Type III (moths, ants, ground beetles, oribatid mites, and collembolas): species richness did not greatly vary with forest age. However, the taxa showing Type III response often had different community compositions among the plots (for soil animals, see Hasegawa et al. 2004). Therefore, uniform species richness did not mean a homogeneous species composition among ages of the stands.

Difference in longicorn assemblage between broad-leaved and conifer stands

A total of 106 longicorn species was collected in Ogawa and Satomi. The average number of species was larger in broad-leaved plots (35.8 SD7.7) in Ogawa than in coniferous ones (22.9 SD8.7) in Satomi (ANOVA, P=0.004). Both in Ogawa and Satomi, species richness was larger in young than in older stands (Fig. 1).

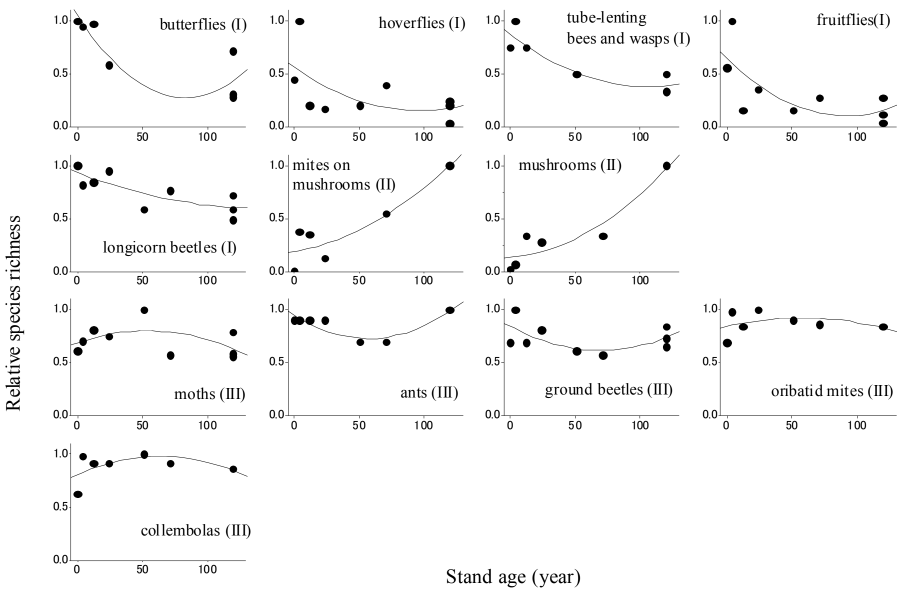


Fig. 1 Relationship between the species richness and the age of regeneration stands after clear-cutting in insects and other organisms in Ogawa, Abukuma area. The notation I, II, or III in parentheses stands for the type of response shown by the particular taxon. The species richness decreases with stand age in Type I, increases in Type II, and does not greatly change in Type III. Lines are quadratic polynomial approximations. Modified from Makino et al. (2006).

The CCA ordination divided the 18 plots into three groups (Fig. 2), which correspond to the following three stand types: initial stage stands, broad-leaved stands, and conifer stands. Species richness was largest in initial stage stands, followed by broad-leaved, and smallest in confer stands; the difference was significant among the three types (ANOVA, P<<0.001) as well as between any pair (corrected for multiple comparison with Bonferroni adjustment).

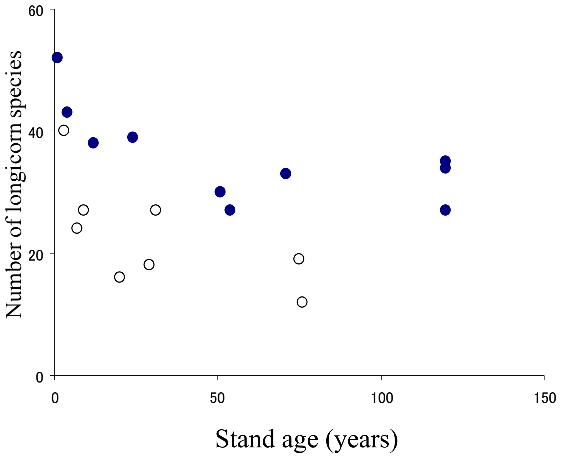


Fig. 2 The number of longicorn species collected with Malaise traps in stands of different ages. Solid and open circles stand for broad-leaved stands and conifer (*Cryptomeria japonica*) plantations, respectively.

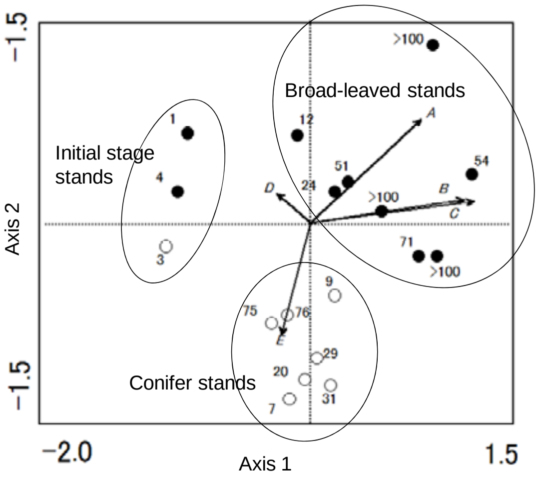


Fig. 3 Ordination of longicorn assemblages by canonical correspondence analysis (CCA). Three forest types are identified. Solid and open circles are as in Fig. 2, and figures show stand ages. Arrows with suffix A to E show variables of forest characteristics, which were significantly related with the division among the three forest groups. See text for more explanation. Modified from Makino et al. (2007).

The CCA analysis selected the following five variables of forest characteristics that are significantly related (P< 0.05) with longicorn species compositions: scores of DCA 2nd axis of forest floor plants (arrow

A in Fig. 3), the maximal DBH of trees (arrow B), the number of species of trees with DBH ≥ 5 cm (arrow C), species richness of forest floor plants (arrow D), and DCA 1st axis of trees with DBH ≥ 5 cm (arrow E). The arrows B and C, both representing the maturation process of forest growth, were nearly parallel to the 1st axis of the CCA diagram (Fig. 3), and explained the discrimination of longicorn species composition between the two older forest types (conifer stands and broad-leaved stands) and the young one (initiation plots). In contrast, the arrows A and E had more vertical directions, and explained the discrimination between broad-leaved and conifer stands. The arrow D representing the species richness of forest floor plants was short, indicating that its effect on the longicorn species composition is marginal.

Discussion

Effects of stand age on insect biodiversity

We showed that the effect of stand age on species richness was not uniform among the taxa monitored. This may be partly due to differential dependence on herbaceous plants. The taxa showing the Type I response, for example, are herbivores during the larval stage, and/or largely depend on flowers for carbohydrates as adults. Many butterfly species live in grasslands and utilize herbaceous plants as hosts. Even species whose larval hosts are trees visit herbaceous plants for nectar as adults (Inoue, 2003). Tube renting bees and wasps probably collect nectar and pollen, or hunt prey (for example, lepidopterous larvae, spiders, and grasshoppers) respectively, in grasslands or in very young and open stands. Although most longicorn beetles feed on dead trees during the larval period, adult insects frequently visit flowers. Then, for taxa showing Type I response, abundance of flowers may be an important factor that contributes to the high species richness observed in plots of early stages of succession. In contrast, those organisms which showed Type II or III responses (mites, soil animals, etc.: Fig. 1) do not seem to require or depend on as abundant a flower resource as do the Type I taxa.

Whatever the underlying mechanisms are, the different responses to the stand age among the taxa indicate that combinations of stands of various ages, or heterogeneously arranged stands, contribute to the maintenance of insect diversity at the landscape level. In Abukuma area, the traditional forestry utilizing regeneration stands by repeating a small-scale clear-cutting has produced open, grassland-like stands as well as middle-aged stands on the one hand, while retaining old forests on the other. This seems to have generally been giving a positive effect on insect diversity.

Effects of conifer plantation on longicorn diversity

The conifer stands, which were monocultures of Japanese cedars, had much smaller numbers of longicorn species than similar-aged broad-leaved stands, except for young plantations (Figs. 3, 4). Probably, the Japanese cedar is not an attractive host plant for longicorns. Kojima and Nakamura (1986) present host records of 468 Japanese longicorn species, of which 30 (6%) feed on (or emerge from) Japanese cedar. However, only two species are recorded as specialists of Japanese cedar or related conifers: the other species have wider host ranges including broad-leaved trees (Kojima and Nakamura 1986). This suggests that a great majority of Japanese longicorn species can live without Japanese cedar, which dominates forested areas in many parts of Japan. On the contrary, a significant decrease in broad-leaved forests and increase in cedar plantations may lead to impoverishment of longicorn faunas, because most species simply have hosts other than Japanese cedar.

An extensive conifer plantation may lead not only to impoverishment of longicorn diversity but to degradation of ecosystem functions that they possibly perform in pollination or in decomposition of dead trees. This is because longicorns frequently visit flowers as adults for pollen and/or nectar feeding, and they principally feed on dead or weakened trees as larvae thus facilitating decomposition by fungi or microorganisms. We urgently need to study the nature and magnitude of conifer plantation on ecosystem functions not only of longicorn beetles but insects in general.

References

Grimaldi D, Engel MS (2005) Evolution of the Insects. Cambridge University Press, New York

Hasegawa M, Fukuyama K, Makino S, Okochi I, Goto H, Mizoguchi T, Sakata T, Tanaka H. (2004) Collembolan and oribatid mites community dynamics with the secondary succession of deciduous forests in Japan. Abstracts of XXII International Congress of Entomology

Inoue T (2003) Chronosequential change in a butterfly community after clear-cutting of deciduous forests in a cool temperate region of central Japan. Entomological Science, 6, 151-163.

Kojima K, Nakamura S (1986) Host tree records of Japanese longicorn beetles. Hiba-Kagaku Kyoiku Shinkosha, Shobara

Makino S, Goto H, Inoue T, Sueyoshi M, Okabe K, Hasegawa M, Hamaguchi, K, Tanaka H, and Okochi I. (2006) The monitoring of insects to maintain biodiversity in Ogawa Forest Reserve. Environmental Monitoring and Assessment, 120: 477-485

Makino S, Goto H, Inoue T, Hasegawa M, Okabe K, Tanaka H, Inoue, T, Okochi I. (2007) Degradation of longicorn beetle (Coleoptera, Cerambycidae, Disteniidae) fauna caused by conversion from broad-leaved to man-made conifer stands of Cryptomeria japonica (Taxodiaceae) in central Japan. Ecological Research, 22: 372-381

Okochi I (2002) A new portable light trap for moth collection. Bulletin of Forestry and Forest Products Research Institute, 1: 231-234

ter Braak CJF, Smilauer P (2002) CANOCO Reference Manual and Canodraw for Windows User's Guide: Software for Canonical Community Ordination (version 4.5). Microcomputer Power, Ithaca

Weisser WW, Siemann E (2004) Insects and Ecosystem Function. Springer, Berlin

Coexistence of Tree Species Based on Structural Diversity

Masahiro Aiba, Tohru Nakashizuka
Graduate School of Life Sciences, Tohoku University

Introduction

Structural characteristics of woody plants, such as plant height, leaf size, and biomass allocation to the aerial parts, are quite variable even among the species in a single community. Some structural variations that are linked to specific functions can be the basis for functional trade-offs and can therefore provide a chance for stable coexistence of plant species, which share many of the resources they consume (Westoby et al. 2002). Since the late 1980s, much effort has been made to elucidate the contribution of structural diversity to the stable coexistence of tree species. These studies have revealed that structural diversity contributes to stable coexistence in various ways. For example, the difference between structures that minimize biomass loss and structures that offer rapid growth is essential for niche differentiation along a spatial light gradient (Walters et al. 1993; Kitajima 1994). Another structural variation related to crown structure and trunk thickness is important in terms of the different strategies adopted by understory shrubs and canopy trees (Aiba and Kohyama 1997; Kohyama et al. 2003). However, past studies have had several weak points, and some critical questions remain unsolved. First, few of these studies have examined the potential for coexistence based on structural variations among ecologically or taxonomically related species, for which competitive exclusion is likely to operate. Second, differences in strategy at the sapling stage have been undervalued. In addition, phylogenetic constraints have rarely been considered in previous studies, even though these constraints could dramatically influence the results (Felsenstein 1985). Given these problems, we attempted to answer two questions: (i) Does the structural diversity of plant body lead to functional trade-offs and stable coexistence, even in taxonomically related late-successional species? (ii) How do structural variations in saplings, and especially variations in crown architecture (Kohyama 1987), contribute to stable coexistence? To do so, we focused on late-successional species in a Bornean rain forest with reference to their molecular phylogeny.

Materials and Methods

The study was conducted at Lambir Hills National Park, Sarawak, Malaysia (4°2′ N, 113°50′ E; 150 m a.s.l.). In the analysis focused on dipterocarp species, we measured the following structural traits: root mass, trunk mass, support-tissue mass, total leaf area, individual leaf area, crown projection area, plant height, and diameter at ground level. For non-dipterocarp species, we measured the same traits except for root mass. For the dipterocarp species, we also recorded the maximum net photosynthetic rates ($A_{\max}$) of saplings under the closed canopy and the wood density of mature trees. $A_{\max}$ was measured in the field in November 2003 and in March 2004 using a portable infrared gas analyzer (LI6400; LI-COR Inc., Lincoln, Nebraska, USA). The wood density of mature trees was obtained from the research literature on tropical timber (PROSEA 1994; Sudou 1970).

For 11 relatively abundant dipterocarp species, we analyzed their demographics using 5×5 m quadrats (248 in total) distributed in a regular matrix that covered 12 ha throughout two permanent sample plots in the park. In June 2003, all juvenile dipterocarps shorter than 2 m that occurred in the quadrats were numbered, their species was recorded, and their height was measured. In June 2004 (1 year after the first census), the quadrats were re-censused, and the status (i.e., living or dead) of all tagged individuals was verified.

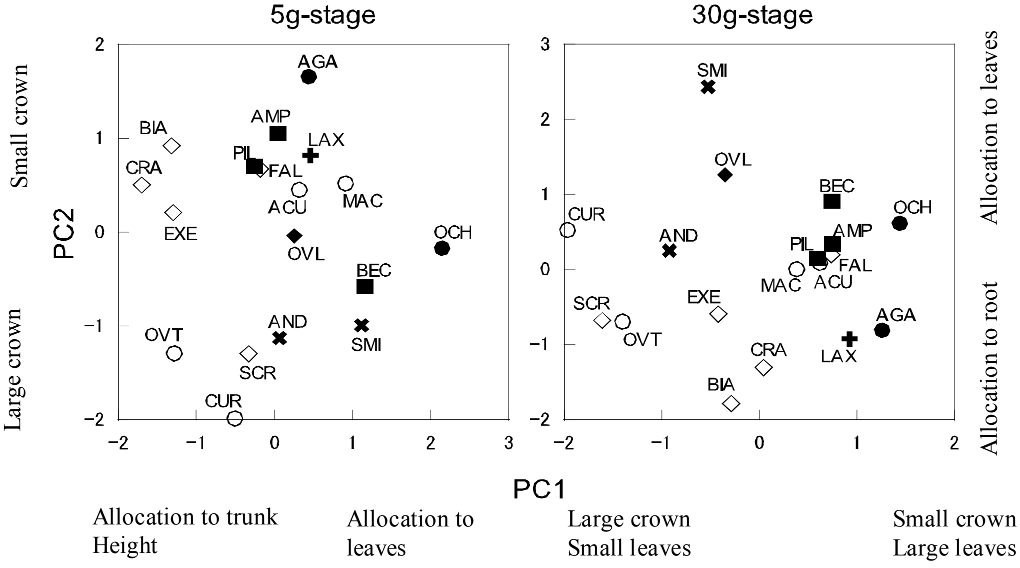
Allometric relationships among the parts of the individual plants were used to describe the structural characteristics of each species at two ontogenetic stages (i.e., with total dry masses of 5 and 30 g). Principal-components analysis (PCA) was performed on the log-transformed estimated values to clarify the constellations of traits by uniting parameters into principal components (PCs). Logistic regression and model selection using the stepwise Akaike information criterion were used to predict the survival rate of juveniles under certain conditions. Using the selected model, survival rates under a canopy openness of 5% at the two ontogenetic stages were estimated. Correlations between structural characteristics and indices of shade tolerance were calculated using the Pearson correlation coefficient for the raw data and, if required, for phylogenetically independent contrasts (Felsenstein 1985).

Results

Sapling structure and regeneration strategy in 18 Shorea species

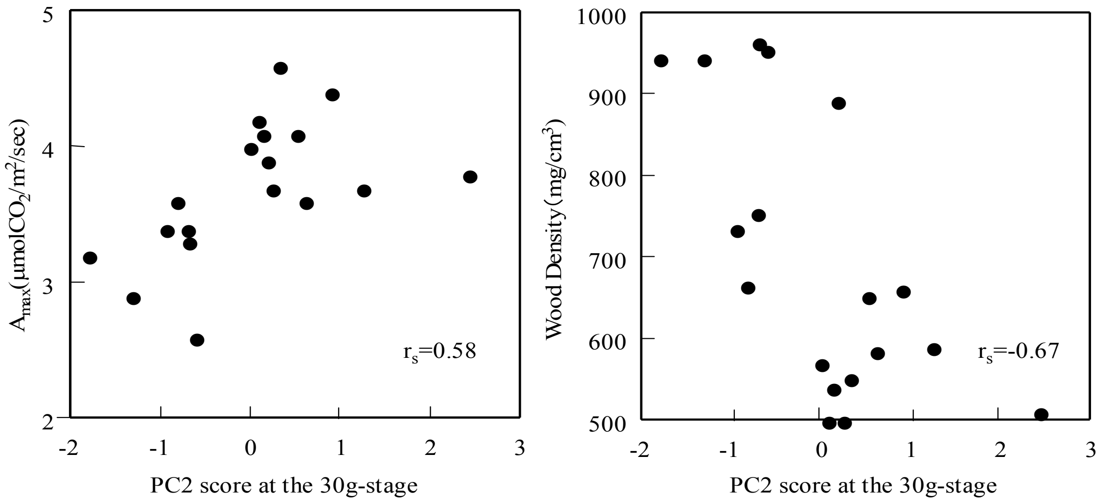
We tested whether the relationship between juvenile tree structure and function is robust among 18 functionally and taxonomically similar sympatric species of genus Shorea in a tropical rainforest in Borneo. The structural variation in saplings of the genus Shorea was as large as that found in sets of species with much more diverse origins. PCA showed that both crown architecture and the biomass allocation to leaves are major sources of structural variation in the 18 investigated species (Fig. 1). Of these two axes, allocation to leaves was significantly correlated with wood density and showed a limited correlation with

Fig.1 Two-dimensional scatter diagrams of ahistorical PCA on the predicted values of morphological traits at two ontogenetic stages. The symbols with plant species codes represent the section of the species; ●: Anthoshorea, ✖: Brachyptera, ○: Mutica, ◆: Ovalis, ■: Pachycarpae, ◇: Shorea.



photosynthetic rate, whereas crown architecture was significantly correlated with seed size (Fig. 2).

Fig.2 Correlation between PC2 score at the 30g-stage and two indices of shade tolerance



Variation in juvenile survival and related physiological traits among 11 late-successional dipterocarp species

To elucidate the environmental factors and physiological traits that most strongly determine the juvenile survival rate, we focused on 11 late-successional dipterocarp species in 248 quadrats distributed throughout a 12-ha area. We monitored the survival of juvenile (height < 2.0 m) individuals of these species for 1 year. The probability of survival for each species was expressed as a logistic function of plant height and three environmental factors: canopy openness, soil-water potential, and the density of conspecific individuals. Seven species exhibited increased survival under more open canopies, and one species exhibited increased survival and two species exhibited decreased survival at drier sites. Six species showed increased survival as the density of their conspecifics increased, whereas two species showed decreased survival under the same conditions. The magnitudes of the effects of the three environmental factors were similar. However, the interspecific difference in survival was most strongly determined by the dependence of small seedlings on their light environment. Survival under low light was positively correlated with root dry mass and negatively correlated with total leaf area of the juveniles. Species that sustained increased mortality under low light had a set of structural traits (i.e., a high root to leaf area ratio) that would potentially allow rapid growth under bright conditions.

Differences in the dry-mass cost of sapling height growth among 56 woody species co-occurring in a Bornean tropical rainforest.

	Cross-species analysis						
	R^2	Adult stature	Crown type	Interaction	Multiple comparison		
					Branched	Simple	Compound
M_W	0.80	**5.26***	1.12	0.55			
M_T	0.80	**8.56****	2.27	0.62			
M_L	0.76	0.96	**3.69***	0.94	ab	a	b
M_S	0.87	1.67	**10.8*****	0.25	a	b	a
M_C	0.74	0.63	1.54	0.71			
A_L	0.71	1.74	1.18	0.58			
A_C	0.71	0.25	1.08	0.49			
A_{IL}	0.94	0.03	**30.3*****	0.70	a	b	b
SLA	0.89	0.08	**5.15****	1.44	ab	a	b
LAI	0.62	1.77	**10.4*****	0.53	a	b	a
D	0.86	**8.59****	**3.63***	0.78	a	b	a
WDI	0.67	0.37	**5.54****	0.33	ab	a	b

Table 2. Summary of two-way ANOVAs with multiple comparisons to test the effect of adult stature and crown type on sapling structural traits. F-values are given for the two main effects and interaction. Degree of freedom of adult stature, crown type and the interaction were 1, 2 and 2. Degree of freedom for residuals were 50 for cross-species analysis. R^2 values were calculated for the model in which the effect of species was nested. Bold values are significant at $P < 0.05$; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. When significant differences were found among crown types, the results of multiple comparisons are also shown. Effects sharing the same letter were not significantly different at $P < 0.05$.

We analyzed the above-ground structure of saplings from 56 sympatric species in our study forest based on their phylogenetic background to elucidate interspecific variation in the dry-mass cost of height growth and its ecological consequences. The estimated extension cost (i.e., the total above-ground mass) for 1.5-m-tall saplings varied by up to 800% among the 56 species. However, no significant differences in extension cost were observed among saplings with three different crown types (branched, monoaxial simple-leaved, and monoaxial compound-leaved; Table 1), even though the monoaxial crown type has been considered to be an adaptation for achieving rapid height growth. The lack of differences have arisen because the advantages of monoaxial saplings in terms of the structural cost for displaying a given leaf area were unexpectedly small. Understory species had a significantly higher extension cost than canopy species because of their thicker (and consequently, heavier) trunks. For all 56 species combined, the trade-off between height growth efficiency and morphology that enhanced light interception (e.g., larger and more numerous leaves) was prominent. This trade-off, which makes efficient vertical growth incompatible with survival in the understory, potentially enables the stable coexistence of these species in a temporally heterogeneous light environment.

Discussion

Our analyses of the dipterocarp species showed that relatively shade tolerant species are characterized by structural traits that minimize biomass loss, such as a larger allocation to roots and a lower leaf mass per unit area, whereas relatively light-demanding species are characterized by structures that promote rapid growth, such as a large allocation to aerial parts and a high leaf area per mass ratio in both the seedling and the sapling stages. Thus, the light gradient can be partitioned by late-successional dipterocarp species even in the absence of canopy gaps based partly on the structural trade-offs, which is similar to the partitioning of the light gradient by pioneer species.

In the 18 Shorea species, structural variation in the saplings could be summarized by two independent axes, an axis for biomass allocation and an axis for crown architecture. This represents the first report that the variation in biomass, which has been discussed mainly for first-year seedlings, is independent of the variation in crown architecture at the sapling stage. In our analysis of 56 tree species, including non-dipterocarps, we revealed an eight-fold variation in extension cost along the axis for variation in crown architecture; therefore, this axis could be correlated with height growth rate. Whereas the variation in biomass allocation enables stable coexistence based on spatial heterogeneity in the light environment, the variation in crown architecture enables coexistence based on temporal heterogeneity in the light environment (Kohyama 1987; Kohyama and Hotta 1990).

The partitioning of spatial and temporal variation of light based on structural diversity and environment-dependent survival of ecologically or taxonomically similar tree species suggests that the community structure is rather stable and deterministic, which contrasts with the prediction of the unified neutral theory of biodiversity and biogeography (Hubbell 2001). Though only a few intensive comparisons of related species have previously been reported, a study of pioneer species in genus Macaranga at the same site also concluded that the coexistence of species was stable and deterministic and was based on interspecific variation in physiology and structure (Davies et al. 1998). Based on the results of our study, we believe that greater efforts to elucidate the mechanisms responsible for the coexistence of ecologically and taxonomically related species will be one of the most effective strategies for providing a thorough understanding of tropical forest communities.

References

Aiba S I, Kohyama T (1997) Crown architecture and life-history traits of 14 tree species in a warm-temperate rain forest: significance of spatial heterogeneity. J Ecol 85:611-624

Davies SJ, Palmiotto PA, Ashton PS, Lee HS, Lafrankie JV (1998) Comparative ecology of 11 sympatric species of Macaranga in Borneo: tree distribution in relation to horizontal and vertical resource heterogeneity. J Ecol 86:662-673

Felsenstein J (1985) Phylogenies and the comparative method. Am Nat 125:1-15

Hubbell SP (2001) The unified neutral theory of biodiversity and biogeography. Princeton University Press, Princeton, New Jersey, USA.

Kitajima K (1994) Relative importance of photosynthetic traits and allocation patterns as correlates of seedling shade tolerance of 13 tropical trees. Oecologia 98:419-428

Kohyama T (1987) Significance of architecture and allometry in saplings. Funct Ecol 1:399-404

Kohyama T, Hotta M (1990) Significance of allometry in tropical saplings. Funct Ecol 4:515-521

Kohyama T, Suzuki E, Partomihardjo T, Yamada T, Kubo T (2003) Tree species differentiation in growth, recruitment and allometry in relation to maximum height in a Bornean mixed dipterocarp forest. J Ecol 91:797-806

PROSEA (1994) Plant resources of South-East Asia 5 (1) Timber trees: Major commercial timbers. Prosea Foundation/Pudoc-DLO, Bogor/Wageningen.

Sudou S (1970) Tropical timbers. Chikyu, Inc., Tokyo.

Walters MB, Kruger EL, Reich PB (1993) Relative growth-rate in relation to physiological and morphological traits for northern hardwood tree seedlings - species, light environment and ontogenic considerations. Oecologia 96:219-231

Westoby M, Falster DS, Moles AT, Vesk PA, Wright IJ (2002) Plant ecological strategies: Some leading dimensions of variation between species. Ann Rev Ecol Syst 33:125-159

Leaf Morphological Distribution in 250 Tree Species in a Lowland Dipterocarp Tropical Rain Forest, Sarawak, Malaysia –Comparison of Homobaric and Heterobaric Leaves

Tanaka Kenzo[1], Tomoaki Ichie[2], Yoko Watanabe[3], Toru Hiromi [4]
[1] *Forestry and Forest Products Research Institute, Tsukuba, 305-8687, Japan*
[2] *Faculty of Agriculture, Kochi University, Nankoku, 783-8502, Japan*
[3] *Hokkaido University Forests, FSC, Sapporo, 060-0809, Japan*
[4] *Ehime Kindergarten, Matsuyama, 790-0003, Japan*

Introduction

Plant leaves show a range of morphological differences among species, including in their shape, size, and structure (Esau 1960). One important characteristic relates to the presence or absence of bundle-sheath extensions (BSEs) in the leaf (Wylie 1952): heterobaric leaves have BSEs, whereas homobaric leaves do not. BSEs are formed by parenchyma or sclerenchyma cells of the vascular bundle sheath, which extend to the epidermis on both sides of the leaf in heterobaric leaves. As a result, the mesophyll of heterobaric leaves is separated into many small “bundle-sheath extension compartments” by the BSEs (Terashima 1992). In contrast, homobaric leaves lack the BSEs and their internal structure is relatively homogeneous. These leaf types differ not only in their structural traits but also in their mechanical and functional characteristics. For example, BSEs in heterobaric leaves may provide mechanical support to the leaf blade (Wylie 1952), may act as a water conduit, or may cause non-uniform photosynthesis (Terashima 1992). Homobaric leaves, in contrast, exhibit larger lateral movements of gases in the leaf than is the case in heterobaric leaves (Pieruschka et al. 2006).

These morphological and functional differences may relate to a tree's growth environment and life form. Some authors have suggested that trees with heterobaric leaves will preferentially be found in deciduous forests, which have dry or cold seasons (Wylie 1952). In contrast, the proportion of trees with homobaric leaves may increase in wet or warm regions, which are usually dominated by evergreen species. This hypothesis is supported by the observation that many more evergreen tree species have homobaric leaves than is the case for deciduous tree species (Wylie 1952). Consequently, in the tropical rain forest, most tree species would be expected to have homobaric leaves, since the forest is humid throughout the year and consists mainly of evergreen trees.

In this study, we hypothesized that leaf type would be correlated with the growth environment or life form rather than with the forest biome, even in a tropical rain forest where most trees have evergreen leaves. The spatial distribution of microenvironmental factors such as light intensity, temperature, and relative humidity varies significantly even within a humid tropical rain forest. In particular, the upper canopy of the forest experiences significant desiccating conditions because of the high light intensities, high temperature, low humidity, and high wind speed, whereas the interior of the tropical rain forest is light-deficient, cooler (as a result of shading), and humid, and has lower wind speeds. Canopy gaps are drier and experience higher irradiance than the forest floor under a closed canopy. As a result of the frequent occurrence of water stress in the canopy and canopy gaps, heterobaric leaves may offer an adaptive advantage even in tropical rain forests. On the other hand, trees with homobaric leaves should be more abundant in forest understory species because of their more humid environment. If the proportion of species with each leaf type varies with the location in such heterogeneous forest environments, the distribution of the two types of leaves may influence stand-level photosynthesis and transpiration traits, which have been reported to differ between leaf types (Terashima 1992). However, only limited information is available on the distribution pattern of the two leaf types and their responses to different growth conditions. In the present work, we investigated the distribution of heterobaric and homobaric leaf types among 250 tree species in 45 families, with different life forms, in a tropical rain forest. In particular, we focused on the relationship between each leaf type and the corresponding life form type (e.g., emergent, canopy, sub-canopy, understory, and canopy gap species).

Materials and Methods

Study site The study was carried out in 2005 in a lowland mixed dipterocarp forest in Lambir Hills National Park, Sarawak, Malaysia (4°12′N, 113°50′E; 150 to 250 m a.s.l.). The mean canopy height was about 30 to 40 m; some emergent trees reached heights of 50 to 70 m. The area has a humid tropical climate, with weak seasonal changes in rainfall and temperature. The mean annual precipitation and temperature were 2540 mm and 26.3 °C, respectively.

Plant material and leaf collection We collected the leaves of 434 individuals of 250 tree species from 127 genera in 45 families, from the forest understory to the emergent layer and in canopy gaps. Between two and five fully expanded and apparently nonsenescing leaves of each species were sampled for microscopic observation. Transverse slices were prepared and photographed under a light microscope. Based on these observations, all tree species were classified into heterobaric and homobaric leaf types (Fig. 1). However, some species displayed intermediate morphology. These leaves had BSEs only around the large veins, and the bundle sheath extension compartments were consequently very large compared with those in heterobaric leaves. In this study, these species were categorized as having homobaric leaves.

All tree species that we studied fell into five categories based on their mature tree height and their distribution bias (canopy gap vs. shaded). The height of mature trees of each species was determined by observation or obtained from the research literature (e.g., Sakai et al. 1999). The tree species were classified into four categories (Table 1) based on their mature height: forest understory (<12.5 m), sub-canopy (12.5 to 27.5 m), canopy (27.5 to 42.5 m), and emergent (>42.5 m). Tree species that grow mainly in canopy gaps were classified in a fifth category (canopy gap species), regardless of their height.

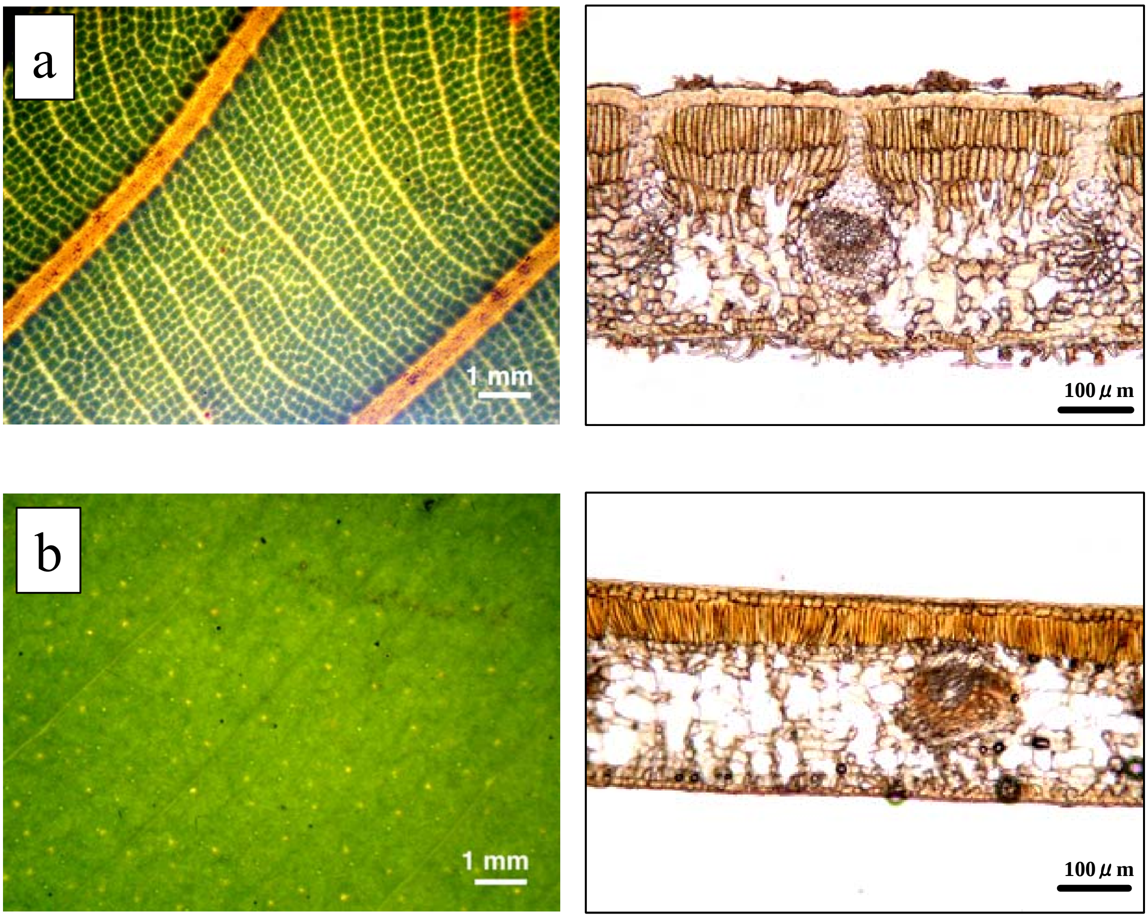


Fig. 1 Photographs of transmission and transverse sections of the leaves.
a: heterobaric leaf (*Lithocarpus luteus*), b: homobaric leaf (*Eugenia subrufa*)

Results

In total, 99 species (40% of the total) in 21 families displayed heterobaric leaves, and 151 species (60%) in 36 families had homobaric leaves (Table 1). The proportion of species with each leaf type differed significantly among life form categories ($P < 0.0001$, χ^2 test). The proportion of trees with heterobaric leaves was only 6% for understory species and 17% for sub-canopy species. The proportion increased to 43% for canopy species and reached 96% for emergent species. The proportion in the canopy gap species was also high (62%)

Table 1 Number of sampled tree species and tree proportion of heterobaric leaf among five life forms

Life form	No. of heterobaric leaf trees	No. of homobaric leaf trees	Total	Heterobaric leaf ratio (%)
Understory	3	46	49	6.1
Subcanopy	13	65	78	16.7
Canopy	23	30	53	43.4
Emergent	47	2	49	95.9
Canopy gap	13	8	21	61.9
Total	99	151	250	39.6

We also found a significant relationship between taxonomic group (family) and leaf type ($P < 0.0001$, $df =$

13, $N = 184$ species; χ^2 test for 14 families that included more than five species in the family). All species of Dipterocarpaceae, consisting mainly of canopy and emergent trees, were classified as heterobaric (see Kenzo et al. 2007). In contrast, species of Annonaceae and Rubiaceae, which appeared mainly in the forest understory, had only homobaric leaves. However, we found seven families (including the Euphorbiaceae and Sapotaceae) that had both leaf types. Species with different leaf types were found even in the same genus in these families: for example, this was true of genus *Macaranga* in the Euphorbiaceae and genus *Santiria* in the Burseraceae.

Discussion

The leaf type of a tree species (heterobaric or homobaric) may depend on its life form type in the tropical rain forest. In general, a greater proportion of evergreen tree species than deciduous species tend to have homobaric leaves (Wylie 1952). Nevertheless, upper canopy and gap species, and especially the most emergent species in the tropical rain forest, had heterobaric leaves, even at the small seedling stage (Table 1).

The difference in the distribution of leaf types as a function of mature tree height may be related to the steep microenvironmental gradient along the tree's height that occurs in a forest ecosystem. Under the canopy conditions of a tropical rain forest, tree leaves of emergent and canopy trees suffer strong desiccating conditions as a result of the higher vapor pressure difference (VPD), temperature, radiation, and winds compared with those in the understory layer (Yoda 1978). The presence of BSEs might confer an advantage to heterobaric leaves over homobaric leaves in the high-water-stress canopy environment. BSEs may be responsible for rapid stomatal response to drought signals, such as a reduction of water potential in the mesophyll or a higher concentration of abscisic acid (ABA), as a result of rapid transportation of these signals via the transpiration stream in BSEs (Terashima 1992). BSEs may also support and protect the leaf blade against collapse after severe dehydration or other stresses and may guide sunlight to thicker sun leaves (Nikolopoulos et al. 2002; Terashima 1992). The relatively high proportion of species with heterobaric leaves in the canopy gap and canopy species may also be related to their dry and sunny environment.

Conversely, the understory of a tropical rain forest is more suited to homobaric leaves, which may perform better than heterobaric leaves under shade. Light intensity in the tropical rain forest decreases significantly with decreasing height below the canopy, usually reaching only a few percent of the level of sunlight above the canopy at the forest floor (Yoda 1978). Under such conditions, the leaves of most plants that reproduce in the understory perform better in the shade. A lack of BSEs may improve the leaf's ability to utilize sunflecks because improved lateral CO_2 diffusion from shaded to illuminated areas of a homobaric leaf will enhance photosynthesis (Lawson and Morison 2006). Homobaric leaves can also increase their proportion of photosynthetically active leaf area. In a study of BSEs in 31 temperate heterobaric leaf tree species, Nikolopoulos et al. (2002) reported that photosynthetically active leaf area decreased from 91 to 48% as the density of BSEs increased. These characteristics of homobaric leaves could thus contribute to improved photosynthetic efficiency under limited light conditions.

The leaf types (heterobaric or homobaric) were also related to the taxonomic groups (family and genus), which usually reflect differences in life form types or growth habitats at or around maturity. Families that mainly appear in the canopy layer, such as Dipterocarpaceae, tend to have heterobaric leaves. In contrast, families that mainly appear in the forest understory, such as Annonaceae and Rubiaceae, tend to have homobaric leaves (see Kenzo et al. 2007). However, families such as Euphorbiaceae and Sapotaceae, which include tree species with a range of life form types from understory to emergent, included both heterobaric and homobaric species. Furthermore, some genera in these families, such as *Macaranga* (Euphorbiaceae), included species with different leaf types. The leaf type in a single species does not change throughout its growth stages or between individuals (Kenzo et al. 2007). These results suggest that the leaf type in each species depends on the growth habitats or life form types at maturity for each species, and these are, in turn, commonly related to the forest microenvironments at maturity.

In conclusion, we found a clear distribution pattern of the heterobaric and homobaric leaf types with respect to the growth environment and life form type of a species, and with respect to taxonomic groups, in a tropical rainforest. Our results suggest that tropical tree species might have adapted to a spatial gradient at maturity in various physical variables, such as light intensity and VPD, by developing different leaf types (heterobaric or homobaric) that offer different physiological and mechanical advantages.

References

Esau K (1960) Anatomy of seed plants. John Wiley and Sons, New York

Lawson T, Morison J (2006) Visualising patterns of CO_2 diffusion in leaves. New Phytol 169:641-643

Kenzo T, Ichie T, Watanabe Y, Hiromi T (2007) Ecological distribution of homobaric and heterobaric leaves in tree species of Malaysian lowland tropical rainforest. Am J Bot 94:764-775

Nikolopoulos D, Liakopoulos G, Drossopoulos I, Karabourniotis G (2002) The relationship between anatomy and photosynthetic performance of heterobaric leaves. Plant Physiol 129:235-243

Pieruschka R, Schurr U, Jensen M, Wolff WF, Jahnke S (2006) Lateral diffusion of CO_2 from shaded to illuminated leaf parts affects photosynthesis inside homobaric leaves. New Phytol 169:779-787

Sakai S, Momose K, Yumoto T, Nagamitsu T, Nagamasu H, Hamid AA, Nakashizuka T (1999) Plant reproductive phenology over four years including an episode of general flowering in a lowland dipterocarp forest, Sarawak, Malaysia. Am J Bot 86:1414-1436.

Terashima I (1992) Anatomy of non-uniform leaf photosynthesis. Photosynth Res 31:195-212

Wylie RB (1952) The bundle sheath extension in leaves of dicotyledons. Am J Bot 39:645-651

Yoda K (1978) Three-dimensional distribution of light intensity in a tropical rain forest of West Malaysia. Malayan Nat J 30:161-177

Changes in Photosynthesis and Leaf Characteristics from Seedlings to Mature Canopy Individuals of Some Dipterocarp Species in a Tropical Rain Forest, Sarawak, Malaysia

Tomoaki Ichie[1], Tanaka Kenzo[2], Yoko Watanabe[3], Reiji Yoneda[2],
Ikuo Ninomiya[4], Takayoshi Koike[3]
[1]*Faculty of Agriculture, Kochi University, Nankoku, 783-8502, Japan*
[2]*Forestry and Forest Products Research Institute, Tsukuba, 305-8687, Japan*
[3]*Hokkaido University Forests, FSC, Sapporo, 060-0809, Japan*
[4]*Faculty of Agriculture, Ehime University, Matsuyama, 790-8566, Japan*

Introduction

Tropical rain forests have a complex and multilayered vertical structure. The crown surface of emergent trees in tropical rain forests usually experiences strong sunlight, whereas less than 1% of the solar radiation typically reaches the forest floor (Chazdon 1988). How do tree leaves respond to such drastic differences in light conditions at differing heights? It is well known that leaves in bright conditions (sun leaves) have greater nitrogen content and leaf mass per unit area (LMA), corresponding to a higher photosynthetic rate at light saturation ($A_{\max}$), than leaves in dark conditions (shade leaves). Shade leaves have a higher chlorophyll content and thinner leaf blade to maintain the dark respiration rate. Thus, the light compensation point (I_c) of shade leaves is smaller than that of sun leaves (Lambers et al. 1998). Carswell et al. (2000) and Rijkers et al. (2000) reported that the $A_{\max}$, LMA, and leaf nitrogen content increased significantly with tree height in some neotropical forest trees, although neither report gave much information about large canopy trees or emergent trees.

Does the photosynthetic capacity of canopy and emergent trees increase with height in the tropical rain forest? Some researchers have reported that the age- and/or size-dependence physiological traits showed an ontogenetic decrease in $A_{\max}$ with decreasing leaf nitrogen content and increasing of LMA in some canopy species (e.g., Thomas and Winner 2002). In general, this decline in leaf nitrogen content causes a reduction in $A_{\max}$ in tall trees (Niinemets 2002). It is well known that $A_{\max}$ may also depend on LMA (Thomas and Winner 2002), and an increase of LMA with tree size leads to an increase in resistance of CO_2 diffusion within the leaf and then a decrease of $A_{\max}$ (Terashima et al. 2001).

Tropical canopy and emergent trees may, however, realize a high $A_{\max}$ by developing a leaf mesophyll structure adjusted to the tropical canopy environment. Kenzo et al. (2004) reported that, in some canopy species with high $A_{\max}$ values (nearly 20 µmol CO_2 m^{-2} s^{-1}) in a Southeast Asian tropical rain forest, $A_{\max}$ had a higher positive correlation with leaf mesophyll structure, such as leaf palisade layer thickness and surface area of mesophyll cells per unit leaf area (A_{mes}/A_a), than with leaf nitrogen content and LMA. Alhough an increase in tree height is usually a known cause of declining physiological function of the hydraulic architecture for the transport of water from roots to leaves, we propose and test the hypothesis that tree height does not limit $A_{\max}$ for tropical canopy species in the tropical rain forest.

Our objective in the present study was to determine the effect of tree height on leaf photosynthesis, as well as on morphological and biochemical properties, in five dipterocarp species. To enable us to sample leaves from seedlings on the dark forest floor to mature canopy trees at the bright canopy layer, we used a canopy crane system that provided three-dimensional access to the forest.

Materials and Methods

Study site and plant material

Our study was conducted in an experimental plot (4 ha, 200×200 m) in a lowland dipterocarp forest in Lambir Hills National Park, Sarawak, Malaysia (4°20'N, 113°50'E; 150 to 250 m a.s.l.) in September 2001. In the study plot, the mean height of the canopy layer in the stand was about 30 to 40 m, and some emergent trees reached 50 m. The annual precipitation and temperature at the study site averaged 2429 mm and 26.3°C from 2000 to 2003, respectively.

We selected five dipterocarp species and 65 individuals, ranging from seedlings to mature trees (Table 1). The species examined were *Dipterocarpus globosus* Vesq. (DG), *Dryobalanops aromatica* Gaertn. f. (DA), *Shorea acuta* Ashton (SA), *S. beccariana* Burck (SB), and *S. macroptera* Dyer (SM). The height of selected trees ranged from 0.6 to 53 m, and the sample included small seedlings, pole-sized saplings, and emergent trees. The seedlings and saplings were chosen both from gaps and their periphery and under a closed canopy.

Gas exchange measurements

Leaf gas exchange rate was measured using a portable photosynthesis apparatus (LI-6400, Li-Cor, Lincoln, NE). All measurements were made in the morning between 0800 and 1100 in order to avoid the midday depression in photosynthesis (Kenzo et al. 2003). We selected three fully expanded and apparently non-senescing leaves taken from the top of the crown. The relation between the photosynthetic photon flux density and the carbon assimilation rate was determined. The light intensity, CO_2 concentration, and temperature in the chamber were controlled at 0 to 1800 µmol photon m^{-2} s^{-1}, 360 ppm, and 30°C, respectively. Based on the measurement data, we estimated A_{max} and I_c.

Leaf nitrogen content, chlorophyll determination, and leaf mesophyll structures

Following the gas exchange measurements, all leaves were sampled, and divided into three parts: one for measuring dry weight and nitrogen content, one for chlorophyll analysis, and the other for observation of mesophyll structure. Leaf nitrogen and carbon contents were determined by an NC analyzer (Sumigraph NC-900, Shimadzu). Chlorophyll was extracted with DMSO and was determined by a spectrophotometer (UV-1400, Shimadzu). The leaves used for observation of the mesophyll structure were fixed in FAA, and transverse slices were prepared. The thicknesses of the leaf blade and palisade layer were determined. The surface area of mesophyll cells per unit leaf area (A_{mes}/A_a) was estimated (Nobel 1999).

Results

Leaf photosynthetic, morphological, and biochemical properties in relation to tree height

Both within and across species, A_{max} and I_c increased significantly with tree height (Fig. 1A, B). We did not find significant interspecific differences in A_{max} and I_c with tree height (ANCOVA; $P > 0.05$), except for in the A_{max} of SB in the canopy individuals.

The leaf blade thickness and LMA increased significantly with tree height (Table 2, Fig. 1C). However, interspecific differences were found between SA and other species. SA had the thickest leaf blade and highest LMA among the species studied at all tree heights (ANCOVA; $P < 0.05$). The thickness of the palisade layer and the A_{mes}/A_a value also increased with tree height without interspecific differences (Table 2, Fig. 1D).

The relationship between nitrogen content per unit area (N_{area}) and tree height was similar to the other traits (Table 2); no significant interspecific difference was observed. The unit mass chlorophyll content (Chl_{mass}) and the chlorophyll to nitrogen (Chl/N) ratio decreased with height without interspecific differences (Table 2).

Leaf mesophyll structure and photosynthetic properties in the canopy

Leaf mesophyll structure significantly differed among species, suggesting a stronger effect on interspecific variation in canopy photosynthesis (Kenzo et al. 2004). In particular, SB leaves had the highest A_{max} (18 μmol m^{-2} s^{-1}) and the thickest palisade layer. Surprisingly, the palisade consisted of up to five or more layers (Fig. 2).

Leaf Amax and Ic in relation to leaf characteristics

Significant correlations were found between A_{max} and LMA and between A_{max} and N_{area} (Table 3; both $P < 0.001$). The highest correlation coefficients were observed between A_{max} and properties of leaf mesophyll structures, such as the thickness of the palisade cell layer and A_{mes}/A_a (Table 3; both $P < 0.001$).

Negative correlations were found between I_c and Chl_{mass} and between I_c and Chl/N (Table 3).

Discussion

Change of photosynthetic capacity and light compensation point with tree height

Photosynthetic capacity (A_{max}) was not limited by tree height in these tropical canopy tree species. For dipterocarp species, A_{max} bears a simple relation to tree height (Fig. 1A). Rijkers et al. (2000) also found a significant relation between A_{max} and tree height in four neotropical species. The slope and intercept of their linear regression line were very similar to those in our study. These facts may be important to estimate the capacity of CO_2 fixation in tropical forests, although further studies are needed in diverse tropical forests.

In contrast, at the dark forest floor, the lower I_c seems to contribute to maintaining a positive carbon assimilation rate (Fig. 1B). I_c was less than 10 μmol photon m^{-2} s^{-1} for most of the non-gap seedlings in this study. Many authors have reported that photosynthetic photon flux density below the closed canopy of tropical rain forests lies in the range of 5 to 20 μmol photon m^{-2} s^{-1} (e.g., Chazdon 1988). In regard to the low I_c for the seedlings, our study species may be well adapted to the low light conditions below the closed canopy of tropical rain forests.

Leaf photosynthetic traits in relation to leaf morphological and biochemical properties

Height-related differences in photosynthetic characteristics such as A_{max} and I_c are closely related to leaf morphological and biochemical traits. It is well known that A_{max} is strongly affected by various leaf characteristics, such as leaf thickness, leaf mesophyll structure (A_{mes}/A_a; Kenzo et al. 2004), and nitrogen content (Evans 1989). In general, age- and size-dependent decline of leaf nitrogen content seems to induce a reduction in A_{max} in tall trees (Koch et al. 2004). However, our results clearly showed that leaf nitrogen content increased with tree height in these tropical canopy species. Although high LMA may also limit A_{max} (Niinemets 2002), dipterocarp canopy trees had a well-developed leaf mesophyll structure, such as a thick palisade layer and high A_{mes}/A_a, which is responsible for reduced leaf internal resistance for CO_2 diffusion, together with high LMA. These results suggest that high leaf nitrogen and a developed mesophyll structure largely contribute to maintaining a high A_{max} in the upper canopy leaves.

The larger Chl_{mass} and Chl/N ratio in the leaves were related to the lower I_c value, permitting better acclimation under dark conditions in the small-tree stage (Table 2). There was a negative correlation between Chl_{mass} and I_c (Table 3), indicating that higher values of Chl_{mass} contribute to increased light harvesting efficiency at lower light availability (Lambert at al. 1998). In our study, the Chl/N ratio of all species increased with decreasing tree height, and the ratio was negatively correlated with I_c (Table 3); these attributes also contribute to improved light harvesting efficiency in darker conditions.

Conclusion

Our results suggest that A_{max} is not limited by tree height in tropical canopy tree species. We also found a simple and significant linear relation with tree height for both leaf photosynthetic characteristics (e.g., A_{max}) and leaf morphological and biochemical traits, which in turn affect photosynthetic traits (e.g., LMA and N_{area}), with some interspecific differences among dipterocarp species. Our study suggests that dipterocarp species can adapt their optimal photosynthetic ability to variable light conditions, from the seedling stage to large adult trees, by changing the morphological and biochemical properties of their leaves.

References

Carswell FE, Meir P, Wandelli EV, Bonates LCM, Kruijt B, Barbosa EM, Nobre AD, Grace J, Jarvis PG (2000) Photosynthetic capacity in a central Amazonian rain forest. Tree Physiol 20:179–186

Chazdon RL (1988) Sunflecks and their importance to forest understory plants. Advanced Ecol Res 18:1–63

Evans JR (1989) Photosynthesis and nitrogen relationships in leaves of C_3 plants. Oecologia 78:9–19

Kenzo T, Ichie T, Ninomiya I, Koike, T (2003) Photosynthetic activity in seed wings of Dipterocarpaceae in a masting year: Does wing photosynthesis contribute to reproduction? Photosynthetica 41:551–557

Kenzo T, Ichie T, Yoneda R, Kitahashi Y, Watanabe Y, Ninomiya I, Koike T (2004) Interspecific variation of photosynthesis and leaf characteristics in five canopy trees of Dipterocarpaceae in a tropical rain forest. Tree Physiol 24:1187–1192

Kenzo T, Ichie T, Yoneda R, Watanabe Y, Ninomiya I, Koike T (2006) Changes in photosynthesis and leaf characteristics with height from seedlings to mature canopy trees in five dipterocarp species in a tropical rain forest. Tree Physiol 26:865–873

Koch GW, Sillett SC, Jennings GM, Davis SD (2004) The limits to tree height. Nature 428:851–854

Lambers H, Chapin III FS, Pons TL (1998) Plant physiological ecology. Springer-Verlag, New York, pp 540

Niinemets Ü (2002) Stomatal conductance alone does not explain the decline in foliar photosynthetic rate with increasing tree age and size in *Picea abies* and *Pinus sylvestris*. Tree Physiol 22:515–535

Nobel PS (1999) Plant Physiology 2nd Edn. Academic Press, NY, pp474

Rijkers T, Pons TL, Bongers F (2000) The effect of tree height and light availability on photosynthetic leaf traits of four neotropical species differing in shade tolerance. Func Ecol 14:77–86

Terashima I, Miyazawa S, Hanba TY (2001) Why are sun leaves thicker than shade leaves? Consideration based on analyses of CO_2 diffusion in the leaf. J of Plant Res 114:93–105

Thomas SC, Winner WE (2002) Photosynthetic differences between saplings and adult trees: an integration of field results by meta-analysis. Tree Physiol 22:117–127

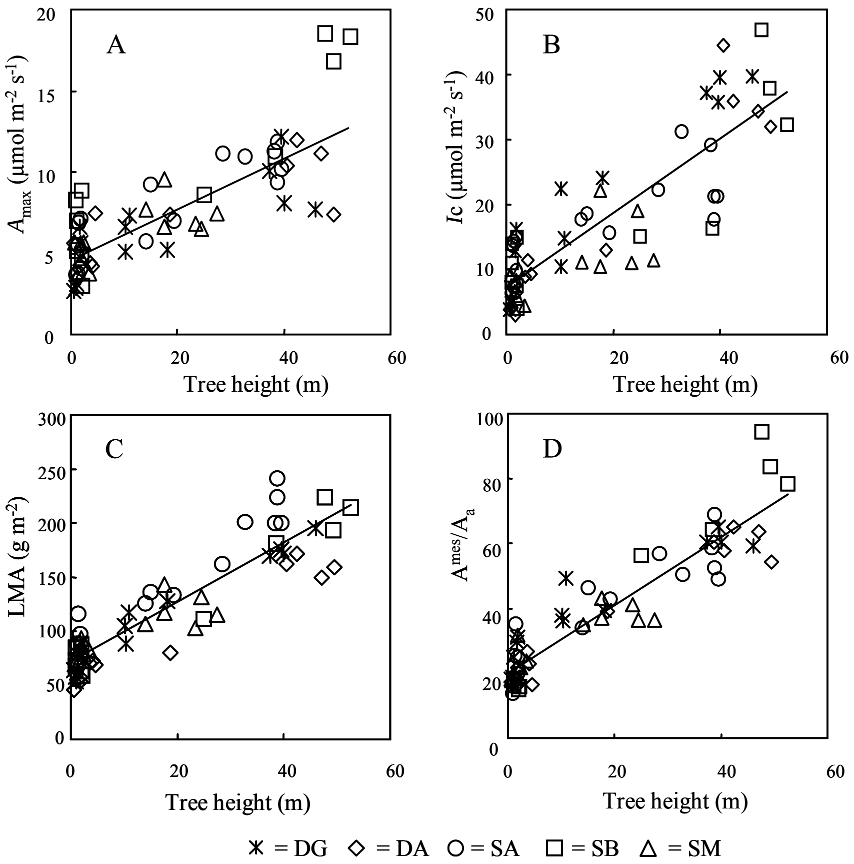


Figure1. Relation between tree height and: A_{max} (A), I_c (B), LMA (C) and A^{mes}/A_a (D) (Kenzo et al. 2006). Values are means for each individual across all replicate leaves. The regression lines are: A, $y = 4.69 + 0.16x$; $r^2 = 0.66$, $P < 0.001$. B, $y = 7.34 + 0.57x$; $r^2 = 0.74$, $P < 0.001$. C, $y = 69.7 + 2.76x$; $r^2 = 0.84$, $P < 0.001$. D, $y = 20.1 + 1.05x$; $r^2 = 0.86$, $P < 0.001$.

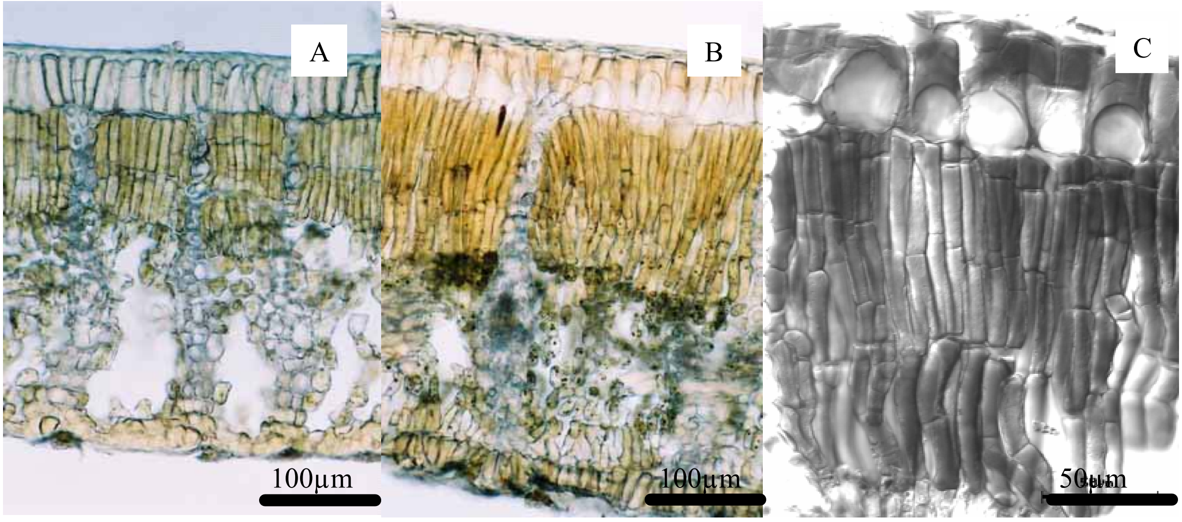


Figure 2. Light micrographs of transverse section of *Dryobalanops aromatica* (A) and *Shorea beccariana* (B). Photograph C is the palisade layer of SB at twice the scale (Kenzo et al. 2004).

Table 1. Tree species, species code, number of individuals, maximum (H_{max}) and minimum (H_{min}) tree height. (Kenzo et al. 2006).

Species	Code	Individual	H_{max} (m)	H_{min} (m)
Dipterocarpus globosus	DG	13	46.0	0.6
Dryobalanops aromatica	DA	12	49.4	0.6
Shorea acuta	SA	15	39.5	1.0
Shorea beccariana	SB	11	52.5	1.0
Shorea macroptera	SM	12	27.5	0.7

Table 2. Relation between tree height and leaf characteristics. Values are means for each individual across all replicate leaves (Data from Kenzo et al. 2006).

Leaf characteristics	Slope	Intercept	r^2	P
Leaf thickness (μm)	4.76	206	0.69	0.001
Palisade layer thickness (μm)	1.97	38.3	0.83	0.001
N_{area} (mol m^{-2})	0.0013	0.045	0.74	0.001
Chl_{mass} (mg g^{-1})	-0.05	3.48	0.49	0.001
Chl/N ratio	-0.06	5.4	0.48	0.001

Table 3. The light-saturated photosynthetic rate (A_{max}) and Light compensation point (I_c) in relation to leaf characteristics. Values are means for each individual across all replicate leaves (Data from Kenzo et al. 2006).

Variable 1	Variable 2	Slope	Intercept	r^2	P
A_{max} (μmol $m^{-2}s^{-1}$)	LMA (g m^{-2})	0.05	1.11	0.63	0.001
	N_{area} (mol m^{-2})	102.9	0.67	0.57	0.001
	Palisade layer thickness (μm)	0.08	1.67	0.76	0.001
	A^{mea}/A_a	0.15	1.85	0.72	0.001
I_c (μmol $m^{-2}s^{-1}$)	Chl_{mass} (mg g^{-1})	-6.98	35.5	0.51	0.001
	Chl/N ratio	-5.06	39.3	0.43	0.001

Functional Diversity of Tree Species in Warm-Temperate Rain Forest Communities of Yakushima Island

Sachi Tanabe[1], Takuya Kubo[1], Shin-ichiro Aiba[2], Takashi Kohyama[1]
[1]*Faculty of Environmental Earth Science, Hokkaido University, Sapporo 060-0810, Japan*
tanabe@ees.hokudai.ac.jp
[2]*Faculty of Science, Kagoshima University, Kagoshima 890-0065, Japan*

Introduction

Natural plant communities are composed of many plant species sharing common traits as autotroph, or competing with each other for common limiting resources. Their coexistence has been examined in terms of differential requirement of multiple resources, spatio-temporal heterogeneity, disturbance-mediated non-equilibrium states and so on. In any situation, it is crucial to describe how species are similar to, or different from each other. From the view of community organization *via* interspecific trade-offs (Silvertown 2004), community response to natural and human disturbance is demonstrated by differential response of species thereby overall ecosystem functioning is maintained efficiently compared to single-species-dominated ecosystems.

We characterize plant species composing warm-temperate rain forests in lowland Yakushima Island from the view of ecomorphological and demographic traits. Yakushima Island located in south from Kyushu Island, Japan, is characterized by well-reserved primary warm-temperate rain forests at around 300-800 m altitude. We show how species differentiation in regeneration process contributes to the resilience of forest ecosystems to various types of perturbation. The within site, and within vegetation type comparison of traits will provide us basic information for among site/type comparison. We paid attention to leaf and seed characteristics in relation to regeneration traits in particular, where detailed examination of leaf longevity with Bayesian analysis is to be presented separately (Kubo, Ushihara and Kohyama, in preparation). In this report we focus on the relationship between seed size, seedling architecture and the choice of regeneration sites among co-occurring warm-temperate rain forest tree species.

Research Sites and Methods

Research sites were set on warm-temperate rain forests in southwestern part of Yakushima Island, mainly watersheds along River Segire, River Ohko and River Koyohji. The detail of the sites can be found in literature (Kohyama 1986, Kohyama and Grubb 1994, Aiba and Kohyama 1996). We use dataset first appeared in these papers.

Roadside census was carried out in the exposed, mountainside berm edge of the Ohko Forestry Road in Ohko Basin. We set two transects, ca. 35 m long and 55 m long with 1 m wide. All saplings taller than 50 cm and less than 2 cm diameter at breast height were monitored, just like the sapling census in nearby Segire River Basin (Kohyama 1986).

Allometry examination was first made at various sites on the southwestern side of Yakushima Island in early July 2006. The unique topography of the warm-temperate forest consists of uniform Miocene granite covering the entire mountain body. Fourteen commonest shade-tolerant tree species were previously studied in a mature closed canopy with no influences of tree-fall gaps (Kohyama and Grubb 1994). 6 tree species frequently seen along roadside banks, 3 of which also observed in shaded forest floors (*Eurya japonica*, *Rhododendron tashiroi*, and *Neolitsea aciculata*), were additionally selected for detailed study. Healthy, well-established seedlings with no breakage, resprouting, or root death, were then carefully excavated with a shovel for minimum root breakage in late October to early November 2006. Seedlings were defined as being less than 30 cm tall; shoot height ranging from 2-27 cm, excluding current-year seedlings. Total sample size summed up to 150 seedlings; 22 to 30 samples for each canopy and subcanopy species. Each seedling was carefully washed and dimensions measured. After dividing the plant into leaves, stems, and roots, each part was photocopied before being dried in an oven at 80 degrees Celsius for two full days. Although the root system was flattened for photocopying, the images were taken so that roots did not overlap with each other so that we could distinguish the actual root distribution. Seventeen species were sampled from habitats at two light conditions (forest floor and exposed road side).

Biomass allocation among root (below-ground) *versus* shoot (above-ground) and that within shoot dimensions (stem *versus* foliage) were analysed using a hierarchical Bayesian model for handling co-occurring all species at once. In this statistical model, factors affecting biomass allocation was categorized into two types— fixed and random effects. We set total seedling weight as the fixed effect that change the mean of the probabilistic distributions, leaving random effects to explain the change in variances dependent on unobservable factors such as unknown or individual seedling characteristics. Four parameters interpreting the fixed effects, deriving from prior distributions based on field data, were used in order to explain and infer the consequences of weight dependency and species difference in biomass allocation of shoot *versus* root and stem *versus* foliage dimensions. The Monte Carlo Markov Chain (MCMC) method was carried out using R2WinBUGS package on R 2.4.1 for acquiring the estimated posterior distributions of parameters. We assume that the probability biomass allocation to shoot p (thus root allocation is $1-p$) would follow the linear logistic model,

$$p = 1/[1+\exp(-z)], \tag{1}$$

where $z = \beta_1 + \beta_2$[log total biomass of seedling]. Equally, stem (q) *versus* foliage ($1-q$) biomass allocation were similarly presented,

$$q = 1/[1+\exp(-z')], \tag{2}$$

where $z' = \beta_3 + \beta_4$[log shoot biomass of seedling]. Each parameter contains a hierarchical structure consisting of a species-specific characteristic and a common characteristic among all 17 species.

Results and Discussion

Figure 1 shows the tendency across 14 shade-tolerant species, examined using dataset by earlier studies. Recruitment capacity is negatively correlated with maximum height (Fig. 1A), that is in accordance with the expected trade-off by the forest architecture hypothesis (Kohyama 1993). Density ratio of canopy gaps to closed stands is higher for shorter species (Fig. 1B), which supports the model implication that lower-layer species show the density spike at a younger stage of community development (T. Kohyama and T. Takada, in preparation).

Figure 2, summarizing the roadside species composition of saplings, suggests that the abundance there

(Fig 2B) is negatively correlated with seed size, but not for the sapling composition in forest gaps (Fig 2A). The two species, *Rhododendron tashiroi* (Rt) and *Eurya japonica* (Ej), are most abundant shade-bearing species in shallow-soil mountainside roadside. The occurrence of such shade-intolerant species *as Aleurites cordata* (Ac) and *Mallotus japonicus* (Mj) was restricted to valley-side road side with deep soil accumulation. When we plot maximum tree height against seed size among 14 shade-bearing species, there was a tendency that tall tree species have large seed size as compared to short tree species (Fig. 3). Such seed mass *versus* maximum adult size correlation is along general trends found in a wide diversity of land plants (Rees and Venable 2007). The dominance of short-stature species in gap-phase regeneration (Fig. 1B) may partly be explained by producing many regenerates (Fig. 1A) with small per-seed size.

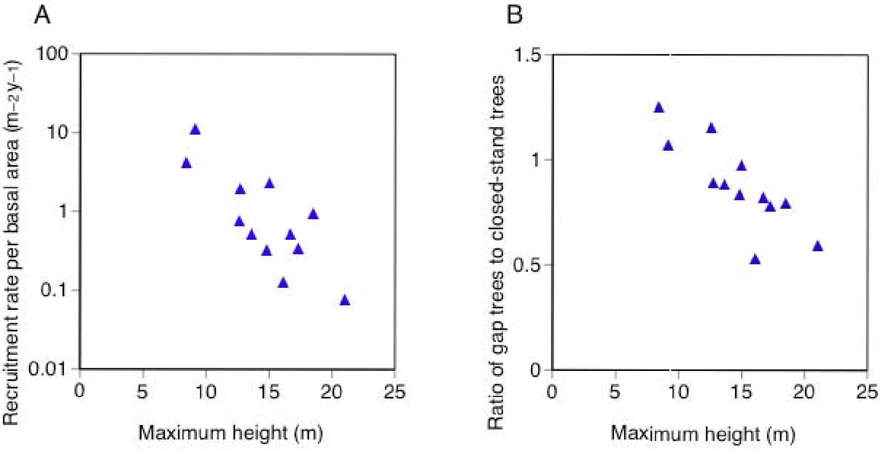


Fig. 1. Interspecific comparison of demographic performance among a dozen abundant shade-tolerant tree species in a primary warm-temperate rain forest in the Segire basin, Yakushima Island, southern Japan. (A) Recruitment rate per species basal area, and (B) ratio of tree densities at canopy gaps to those at closed-canopy stands, plotted against observed maximum height. Density and recruitment rate are defined above 2-cm trunk diameter at breast height. Based on census data of permanent plots monitored since 1981 (Kohyama 1986; Aiba and Kohyama 1996).

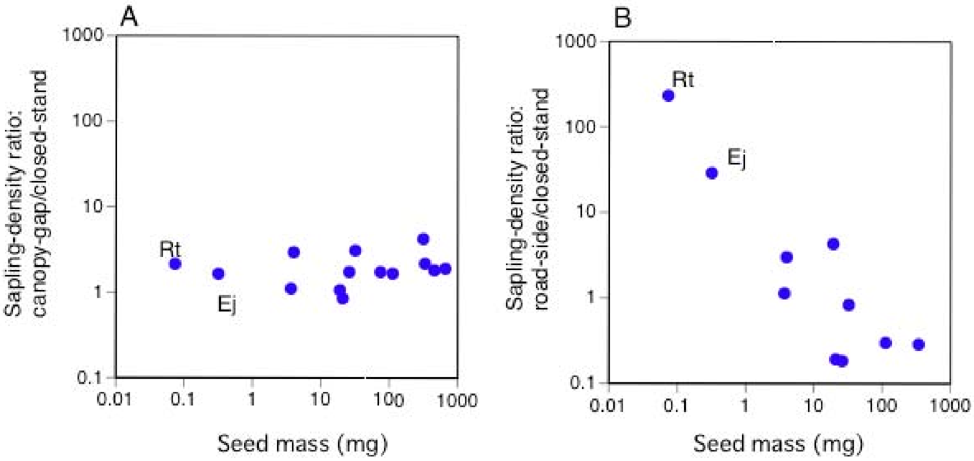


Fig. 2. Ratio in density of saplings (> 50 cm tall and < 2 cm dbh) between tree-fall gaps and closed stands (A), and that between roadside rocky banks and closed stands (B) for 14 abundant shade-tolerant species of warm-temperate rain forest in Yakushima, plotted against dry weight of seeds. Studied area is 2000 and 650 m^2 for closed stands and gaps (in Segire River basin), respectively, and is 90 m^2 for roadside banks (in Ohko River basin).

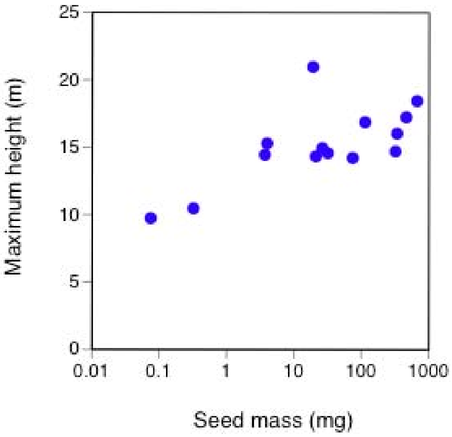


Fig. 3. Relationship between seed size and maximum attainable tree size of common 14 abundant shade-tolerant species in Segire River basin of Yakushima Island.

Two combinations of biomass allocation patterns among species were examined: (1) allocation patterns between root and shoot parts and (2) those within shoot, i.e. stem and foliage. In root versus shoot allocation, results showed that an average-sized individual, ignoring interspecific differences, would allocate resources to shoot biomass (Fig. 4). Species difference was found for eight species including large-seeded *Camellia sasanqua* (Cs) and small-seeded *Eurya japonica* (Ej), where significant species difference was recognized for those that do not include the mean of hyperspecies posterior distribution within the 95% confidence interval. When examining only the differences among species, *Eurya japonica* (Ej), *Cleyera japonica* (Cl), and *Litsea acuminata* (La) showed a positive response to shoot allocation within all 17species. *Stewartia monadelpha* (Sm), *Aleurites cordata* (Ac), *Camellia sasanqua* (Cs), *Neolitsea aciculata* (Na) and *Acer morifolium* (Am), on the other hand, showed root allocation to be more significant. Overall, there was no significant dependency on total dry mass allocation with change in seedling size.

When comparing stem to foliage allocation, as Fig. 5 shows, allocation in shoot dimensions showed to slightly invest in foliage biomass. *Eurya japonica* (Ej) and *Symplocos tanakae* (St) were “foliage-investing” species, while two deciduous, shade-intolerant species *Stewartia monadelpha* (Sm) and *Aleurites cordata* (Ac) were classified as “stem-investing” species. Results showed that stem-investing species allocated biomass in roots, while foliage-investing species *Eurya japonica* was a shoot-allocating species when root-shoot biomass allocation was compared. No species difference was found for foliage dry mass dependency in stem *versus* foliage biomass allocation.

A distinctive interspecific difference in biomass allocation and its dependency on total dry mass or shoot dry mass in root *versus* shoot and stem *versus* foliage allocation patterns, respectively, was not established from these results. However, architectural differences in species were visible among species from observations. *Rhododendron tashiroi*, for example, developing shallower horizontal roots compared to other shade-tolerant species, nevertheless it did not show a significant trait in biomass allocation patterns. It can thus be considered that factors other than biomass allocation would be involved with regulation in seedling architectural performance related to species diversity; even among root-allocating species, difference may occur between seedling height *versus* width dimensions. Results regarding biomass-allocating patterns would simply characterize one aspect of species' architecture; it would be insufficient in extensive comparison of species' architecture. Further analysis therefore, including architectural dimensions comparing above-ground and below-ground, such as depth *versus* height and crown *versus* root width, within above-ground and below-ground architectures will be necessary. It is also interesting to note that small-seeded species such as *Eurya japonica* and *Rhododendron tashiroi* and large-seeded species *Aleurites cordata* were both commonly seen along rocky roadside banks. While hypothesized that seedling architecture is correlated with seed size and its choices in sites for establishment, the classical proposal of the relationship between seed size and shade-tolerance may not directly apply for species studied here.

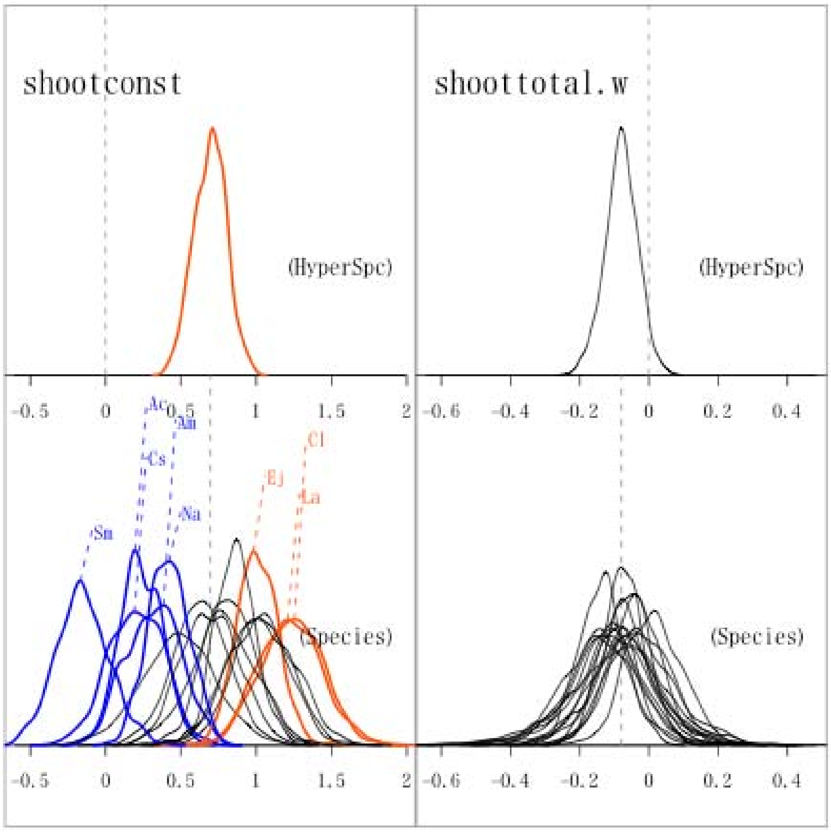


Fig. 4. Biomass allocation among root and shoot. The posterior distribution results of the hierarchical Bayesian model, each parameter expressed with 2 distribution panels. Vertical axes indicate frequency of distributions for posterior probabilities. Top panels, parameter distributions considering a common characteristic among all species (hyperspecies). Parameters which do not include a mean value of 0 within the confidence interval are shown in following colors; red as to those distributing in positive probabilities, blue for those in negative, black for those including the mean value within the confidence interval. Bottom panels, parameter distributions considering species difference, each line representing a species. Colors are sorted accordingly to the mean value derived from the posterior hyerspecies distribution. Panels on the left show results for constant term (β_1) for shoot *versus* root allocation patterns. Panels on the right show total mass dependency (β_2), in eqn (1).

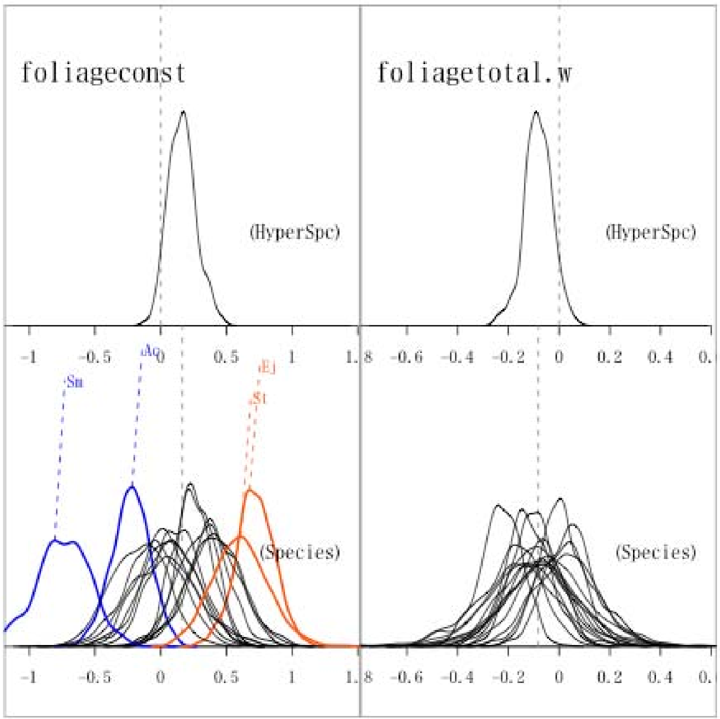


Fig. 5. Allocation patterns in stem and foliage. The posterior distribution results of the hierarchical Bayesian model, each parameter expressed with 2 distribution panels. Vertical axes indicate frequency of distributions for posterior probabilities. Top panels, parameter distributions considering a common characteristic among all species (hyperspecies). Parameters which do not include a mean value of 0 within the confidence interval are shown in following colors; red as to those distributing in positive probabilities, blue for those in negative, black for those including the mean value within the confidence interval. Bottom panels, parameter distributions considering species difference, each line representing a species. Colors are sorted accordingly to the mean value derived from the posterior hyerspecies distribution. Panels on the left show results for constant term (β_3) for foliage allocation patterns. Panels on the right show shoot mass dependency (β_4), in eqn (2).

Acknowledgments

We greatly appreciate Fumihiro Iwagawa for his support and suggestion on field works.

References

Aiba S, Kohyama T (1996) Tree species stratification in relation to allometry and demography in a warm-temperate rain forest. J Ecol 84:207-218

Kohyama T (1986) Tree size structure of stands and each species in primary warm-temperate rain forests of southern Japan. Bot Mag Tokyo 99:267-279

Kohyama T (1987) Significance of architecture and allometry in saplings. Funct Ecol 1:399-404

Kohyama T (1993) Size-structured tree populations in gap-dynamic forest - the forest architecture hypothesis for the stable coexistence of species. J Ecol 81:131-143

Kohyama T, Grubb PJ (1994) Below- and above-ground allometries of shade-tolerant seedlings in a Japanese warm-temperate rain forest. Funct Ecol 8:229-236.

Rees M, Venable DL (2007) Why do big plants make big seeds? J Ecol 95:925-936

Silvertown J (2004) Plant coexistence and the niche. Trends Ecol Evol 19:605-611

Reproductive Strategy of a Tropical Pioneer Shrub, *Melastoma malabathricum* (Melastomataceae)

Kuniyasu Momose [1], Tamaki Kamoi[1], Tanaka Kenzo[2], Koichiro Kuraji[3]
[1] *Faculty of Agriculture, Ehime University, Japan*
[2] *Forests and Forest Products Research Institute, Japan*
[3] *University Forest in Aichi, The University of Tokyo, Japan*

Introduction

Many studies of excess flower production (the production of more flowers than fruits) have been conducted. Holtsford (1985) described five categories of hypotheses to explain this phenomenon: male function, pollinator attractiveness, reproductive assurance, resource boom, and selective fruiting.

The resource boom hypothesis assumes that the amount of resources that will be available for fruit production is not predictable at the flowering stage and that high fruit set occurs only when the resources for fruit production are sufficient. Previous studies of this hypothesis dealt with the unpredictability of supra-annual environmental fluctuations (Holtsford 1985; Eriksson 1987; Gorchov 1988; Vaughton 1991; Nishikawa 1998). However, in tropical regions, some pioneer plants reproduce continuously (Momose et al. 1998; Sakai et al. 1999), and they are considered to be affected by environmental fluctuations over much shorter time scales.

Previously, the resource boom hypothesis has been tested under artificial environmental control (Coulter 1979; Willson and Price 1980; Lee and Bazzaz 1982; Holtsford 1985; Eriksson 1987; Gorchov 1988; Vaughton 1991; Nishikawa 1998). As a new approach to testing this hypothesis, we measured fluctuations in the demand and supply of resources under natural conditions in a species that reproduces continuously and clarified how plants adapt to those fluctuations through increasing the number of reproductive organs and aborting these organs when resources are insufficient.

Methods

Study site and plant materials

The study was conducted in Lambir Hills National Park (4°12′N, 114°02′E; ca. 100 m a.s.l.), Sarawak, Malaysia. The climate is almost aseasonal (Kato et al. 1995).

The study species, *Melastoma malabathricum* L., is a pioneer shrub that is abundant along the edges between natural and artificial habitats (e.g. beside roads, Corner 1988). These plants reproduce almost continuously throughout the year, and reproductive organs at various stages, including young flower buds and mature fruits, are almost always found on a given individual.

Size and dry weight

To estimate the dry weight of the reproductive organs attached to plants, we determined relationships between their size and weight. To create these relationships, we harvested and measured 94 buds, 21 flowers, and 71 fruits (parameters: fresh weight, dry weight, length, and width). From these samples, we performed multiple regressions to analyze the relationships between dry weight (the explained variable) and length and width (independent variables) and between the water content in the reproductive organs (water weight) and their size (length and width).

Growth of reproductive organs

From 5 June until 24 July 2004, we measured the lengths and widths of marked reproductive organs every day without damaging the organs. In total, we measured the growth of 90 samples from six plants. The abortion rate of reproductive organs (the proportion of organs originally present on one day that were no longer present on the next day) was obtained from these samples. The size growth of the reproductive organs was transformed into a dry weight growth using the multiple regression equations for dry weight as a function of size. We calculated water weight similarly.

Monitoring the number of reproductive organs

We monitored fluctuations in the number of reproductive organs on each individual between consecutive days. The number of reproductive organs was counted daily in each of seven categories based on their reproductive stage: bud (young, intermediate, large), flower, and fruit (young, intermediate, and large). These stages were defined for the reproductive organs according to their sizes and colors.

Respiration and transpiration of reproductive organs

Diurnal changes in respiration and transpiration were measured every hour during the day and every 3 hours at night using an LI-6400 portable photosynthesis meter (LI-COR, Lincoln, Nebraska) with a model 6400-05 conifer chamber. In total, 56 samples (nine individuals) from seven reproductive stages were measured on nine individual plants. Under natural light, each sample was measured for only 1 day, and then its dry weight was determined.

Photosynthesis of leaves

Diurnal changes in leaf photosynthesis were measured using the LI-6400 meter under natural light conditions. After the measurements, the area of each leaf was measured, and the area of each leaf was calculated.

Meteorological data

Solar radiation was measured at 10-minute intervals using a Pyranometer (MS-801, EKO Seiki) at a fixed open site. The plants in our study were assumed to receive approximately the same amount of solar radiation as the sensor because they were located at the roadside. Rainfall was measured every hour using a tipping-bucket rain gauge that collected 0.5 mm per tip (No.34-T, OTA).

Analysis

Based on the assumption that carbohydrates and water were demanded by reproductive organs and that their supply fluctuated in the short term, we analyzed the responses of plants as a function of these fluctuations.

Demand for carbohydrates was expressed as the increase in dry weight (g), and carbohydrate consumption was expressed as the respiration per reproductive organ per day (g $C_6H_{12}O_6$ day^{-1}). Similarly, demand for water was expressed as the increase in water weight (g) and water consumption of the transpiration per reproductive organ per day (g H_2O day^{-1}). The average demands for carbohydrates and water at each stage were thus obtained. The number of reproductive organs counted at each reproductive stage on each day was multiplied by the carbohydrate and water demands for that stage to produce an estimated total demand for carbohydrates and water for nine plants each day.

The resource supply was determined from our photosynthetic measurements and meteorological data. The relationships between photosynthetic photon flux density (PPFD) and photosynthetic rate were estimated from the gross photosynthetic rate, the maximum gross photosynthetic rate, the respiration rate, and PPFD (μmol m^{-2} s^{-1}; Boote and Loomis 1991). The relationship between total radiation and PPFD at our study site was provided by Megumi Yamashita (Kinki Surveyor School, personal communication). The radiation measured on each day from 31 May to 17 July 2004 was transformed into PPFD based on this conversion factor. The daily total photosynthetic productivity (g cm^{-2} day^{-1}) was estimated for each day as a function of the calculated PPFD values. The daily total rainfall was also calculated.

The daily abortion rate of the population on the next day was compared with the carbohydrate demand on the current day divided by the total photosynthetic productivity during the previous 1, 3, or 5 days and with the demand for water on the current day divided by the total rainfall during the previous 5 days. Spearman's rank correlation coefficient was calculated for each of these comparisons.

Results

The demand for resources (carbohydrates and water) was greatest during the flower stage (Table 1). The photosynthetic rates of leaves and PPFD both varied during the day. Because stomatal conductance decreased after 1000, we obtained separate relationships between PPFD and photosynthetic rate before and after this time. Maximum gross photosynthetic rates of leaves before and after 1000 were 11.0 and 7.3 μmol CO_2 m^{-2} s^{-1}, respectively. The respiration rate was 0.80 μmol CO_2 m^{-2} s^{-1}. The leaf transpiration rate averaged 376 mg cm^{-2} day^{-1}, and the mean area of a single leaf was 19.7 cm^2.

The daily abortion rates on the next day were significantly correlated with the carbohydrate demand on the current day divided by the total photosynthetic production per unit leaf area during the previous 1, 3, or 5 days (Table 2). However, the daily abortion rates on the next day were not correlated with the water demand on the current day divided by the total rainfall during the previous 5 days. That is, the daily abortion rates were correlated with the available carbohydrate resources but not with the available water resource.

Discussion

The daily abortion rates were correlated with the carbohydrate demand divided by the total photosynthetic production per unit leaf area during the previous 1, 3, or 5 days. The abortion of reproductive organs in *M. malabathricum* thus appears to represent an adaptation to environmental fluctuations at shorter time scales than previously reported in other plants.

Even in the absence of seasonal environment limitations, tropical climax species tend to reproduce at intervals of 1 year or longer (Momose et al. 1998; Sakai et al. 1999). This suggests that they reserve resources, and that the higher their photosynthetic production, the more their resource reserves increase (Yoneda et al. 2002). In contrast, tropical pioneer shrubs such as *M. malabathricum* reproduce continuously. When photosynthetic production is low, they abort their reproductive organs. Such a reproductive strategy, which minimizes the resources reserved for reproduction, can be explained by the typically high mortality of tropical pioneer species (Clark and Clark 1992, Shimano 2000).

Acknowledgments

We thank Tomoaki Ichie, Kochi University, for his support; Megumi Yamashita, Kinki Surveyor School, for permission to use unpublished meteorological information; Takayoshi Koike, Hokkaido University, for loaning us a conifer chamber; and Tomonori Kume, University of Tokyo, for help with meteorological studies. This study was carried out as part of project #2 of the Research Institute of Humanity and Nature organized by Tohru Nakashizuka, and the CREST project organized by Masakazu Suzuki, University of Tokyo. We thank the organizers of these projects and their Malaysian counterparts, Lucy Chong, Sarawak Forest Corporation, and Josef J. Kendawang, Sarawak Forestry Department, as well as other project members.

References

Boote KJ, Loomis RS (1991) The prediction of canopy assimilation. In: Boote KJ, Loomis RS (eds) Modeling crop photosynthesis – from biochemistry to canopy. Crop Science Society of America, Madison, Wisconsin USA, pp 109-140

Clark DA, Clark DB (1992) Life history diversity of canopy and emergent trees in a neotropical rain forest. Ecological Monographs 62: 315-344

Corner EJH (1988) Wayside trees of Malaya 3rd ed. The Malayan Nature Society, Kuala Lumpur, Malaysia

Coulter MC (1979) Seed abortion in *Anagallis arvensis* on southeast Farallon Island, California. Madroño 26:101-102

Eriksson O (1987) Regulation of seed-set and gender variation in the hermaphroditic plant *Potentilla anserine*. Oikos 49:165-171

Gorchov DL (1988) Effects of pollen and resources on seed number and other fitness components in *Amelanchier arborea* (Rosaceae: Maloideae). Am J Bot 75:1275-1285

Holtsford TP (1985) Nonfruiting hermaphroditic flowers of *Calochortus leichtlinii* (Liliaceae): potential reproductive functions. Am J Bot 72:1687-1694

Kato M., Inoue T, Hamid AA, Nagamitsu T, Merdek MB, Nona AR, Itino T, Yamane S, Yumoto T (1995) Seasonality and vertical structure of light-attracted insect communities in a dipterocarp forest in Sarawak. Res Popul Ecol 37:59-79

Lee TD, Bazzaz FA (1982) Regulation of fruit and seed production in an annual legume, *Cassia fasciculata*. Ecology 63:1363-1373

Momose K, Ishii R, Sakai S, Inoue T (1998) Plant reproductive intervals and pollinators in the aseasonal tropics: A new model. Proc Roy Soc London B 265:2333-2339

Nishikawa Y (1998) The function of multiple flowers of a spring ephemeral, *Gagea lutea* (Liliaceae), with reference to blooming order. Can J Bot 76:1404-1411

Sakai S, Momose K, Yumoto T, Nagamitsu T, Nagamasu H, Hamid AA, Nakashizuka T (1999) Plant reproductive phenology over four years including an episode of general flowering in a lowland dipterocarp forest, Sarawak, Malaysia. Am J Bot 86:1414-1436

Shimano K (2000) A power function for forest structure and regeneration pattern of pioneer and climax species in patch mosaic forests. Plant Ecology 146: 207-220

Vaughton G (1991) Variation between years in pollen and nutrient limitation of fruit-set in *Banksia spinulosa*. J Ecol 78:389-400

Willson MF, Price PW (1980) Resource limitation of fruit and seed production in some *Asclepias* species. Can J

Bot 58:2229-2233

Yoneda R, Ninomiya I, Patanaponpaiboon P, Ogino K (2002) Carbohydrate dynamics on [sic] trees in dry evergreen forest, Thailand. I. Diel fluctuation of carbohydrates in a whole tree of *Hydnocarpus ilicifolius*. Tropics 11:125-134

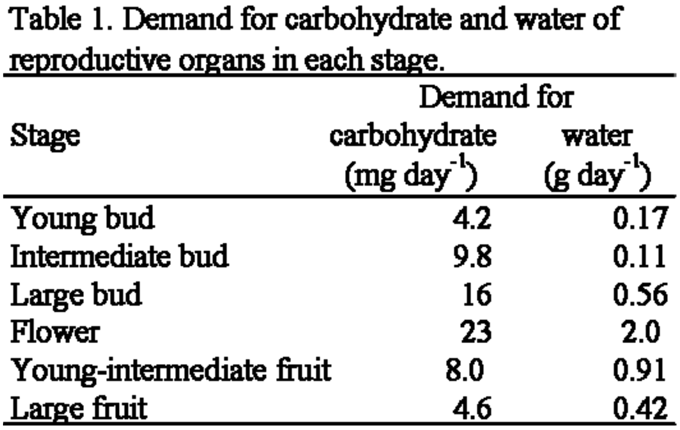


Table 1. Demand for carbohydrate and water of reproductive organs in each stage.

Stage	Demand for carbohydrate (mg day^{-1})	water (g day^{-1})
Young bud	4.2	0.17
Intermediate bud	9.8	0.11
Large bud	16	0.56
Flower	23	2.0
Young-intermediate fruit	8.0	0.91
Large fruit	4.6	0.42

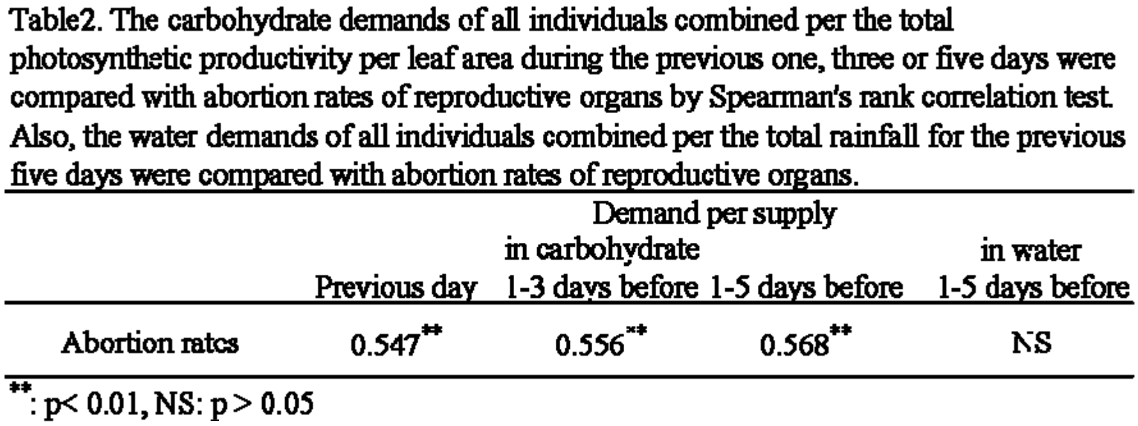


Table2. The carbohydrate demands of all individuals combined per the total photosynthetic productivity per leaf area during the previous one, three or five days were compared with abortion rates of reproductive organs by Spearman's rank correlation test. Also, the water demands of all individuals combined per the total rainfall for the previous five days were compared with abortion rates of reproductive organs.

	Demand per supply in carbohydrate: Previous day	1-3 days before	1-5 days before	in water: 1-5 days before
Abortion rates	0.547**	0.556**	0.568**	NS

**: $p < 0.01$, NS: $p > 0.05$

Variations in the Quantity of Attractants in Floral Odors and their Effects on Beetle Pollinator Arrivals in *Homalomena propinqua* (Araceae)

Yuko Kumano, Ryohei Yamaoka
Graduate School of Science and Technology, Kyoto Institute of Technology

Introduction

The Araceae represent one of the most diverse and abundant families in the humid tropics (Young 1986; Mayo et al. 1997), and their inflorescences are usually monoecious and protogynous (Bernhardt 2000). Beetle pollinators tend to arrive more frequently at the pistillate-phase inflorescences during the period of heat generation by the spadices than at the staminate-phase inflorescences, and this difference in timing can promote cross-pollination (Gibernau et al. 1999; Bernhardt 2000).

Homalomena propinqua (subfamily Aroidae) is a common understory herb found in lowland dipterocarp forests in Borneo, and two types of beetles (*Parastasia bimaculata* Guerin, Scarabaeidae; *Dercetina* sp., Chrysomelidae) are its main pollinators (Kato 1996; Momose et al. 1998; Kumano and Yamaoka 2006). Protogynous inflorescences of this species remain open for 3 days; the spathe is fully open by 0500 on the first day, and adhesive pollen begins to be shed on the morning of the second day. Beetle pollinators arrive at inflorescences mainly between 0700 and 1000, when the spadices generate endothermic heat (Kumano and Yamaoka 2006). Pollinators remain in the floral chamber with eating and/or mating, until the spathe closes tightly on the morning of the fourth day.

In our previous study (Kumano and Yamaoka 2006), we showed that the quantity of attractants released by the plant in the floral odors increased during the period of endothermic heat generation and proposed that the arrival of beetle pollinators may be affected by the increase in the quantity of these attractants. However, little information has been published about the attractiveness of floral odors even though beetle pollinators are attracted over long distances mainly by olfactory cues (Gottsberger and Silberbauer-Gottsberger 1991). In this study, we focused on variations in the quantity of attractants in floral odors to elucidate the relationship between quantity of attractants and arrivals of beetle pollinators in the *H. propinqua* pollination system. To do so, we first observed beetle pollinator arrivals during each floral stage, collected and analyzed floral odors, and then we conducted a bioassay of the main volatiles contained in these floral odors. We also investigated the relationships between variations in the quantity of attractants and the frequency of pollinator arrivals both during heat generation by the spadices and among floral stages. We use the results of these analyses to discuss the factors responsible for the reproductive success of *H. propinqua.*

Materials and Methods

Study site and materials

Our study was carried out in a lowland dipterocarp forest in Lambir Hills National Park, Sarawak, Malaysia (4°20′N, 113°50′E; 150 to 250 m a.s.l.). We found two *H. propinqua* populations along small streams, located 100 m apart and separated by a ridge. All examinations were conducted using these two populations.

Observation of pollinator arrivals during each floral stage

To determine the frequency of beetle pollinator arrivals during each floral stage, we used unpollinated inflorescences (40 inflorescences on the first day of flowering, 44 inflorescences on the second day, and 19 inflorescences on the third day). All inflorescences were covered with polyethylene bags the day before flowering and remained covered until our observations began. Bags were removed at 0600, and pollinators were then free to arrive at the inflorescences. Thereafter, we caught pollinators inside the floral chambers at 1030 hours using polyethylene bags. Differences in pollinator arrivals were compared using a two-way ANOVA followed by Schéffe's multiple-comparison test.

Bioassays using the main compounds found in floral odors

Bioassays were conducted using laboratory standard chemicals for the five main components of floral odor in *H. propinqua*, which together represented 88% of the floral odors compositions by mass on the first day(Kumano and Yamaoka, 2006). To test their effectiveness, we added 100 µg per hour of each test compound on a Petri dish placed below a plant with no inflorescences. We recorded the species and the total number of pollinators that flew in a zig-zag pattern above the Petri dish or that landed on the dish between 0630 and 1030. As a negative control, we used an empty Petri dish placed beside the Petri dish containing the test compound. As a positive control (intact flowers), we counted the total number of beetles that remained in open flowers once per week when we conducted the bioassays. To confirm differences in attractiveness of pollinators between test compounds and positive controls (intact flowers), we transformed the number of individuals to log $(\chi + 1)$ scale and subjected the resulting data to a one-way ANOVA, followed by Dunnet's test.

Sampling of floral odors

To examine temporal variation in the quantity of attractants during the morning pollination period, we used eight unpollinated inflorescences per day from the first day to the third day of flowering. To confirm the variation in attractant quantities among floral stages, we used 10 unpollinated inflorescences and sampled consecutively from the first to the third day of flowering between 0700 and 0800 hours, when both of the main beetle pollinators visit most frequently (Kumano and Yamaoka, 2006). The methods used for collection and chemical analyses of the floral odors are described by Kumano and Yamaoka (2006). Temporal variations in the quantity of attractants were analyzed using the Kruskal-Wallis test combined with Scheffé's test. Differences among floral stages were tested using Friedman's test combined with a Steel-Dwass test.

Results

Observation of pollinator arrivals at each floral stage

The mean number of pollinator arrivals at each floral stage is shown in Fig. 1. The mean number of P. bimaculata arrivals decreased significantly from day 1 to day 3 (Schéffe's test, $P < 0.01$). On the other hand, the mean number of Dercetina sp. arrivals showed no significant differences among floral stages ($P > 0.05$).

In addition, the number of P. bimaculata arrivals on the third day was significantly lower than the number of Dercetina sp. arrivals at every floral stage (P < 0.05).

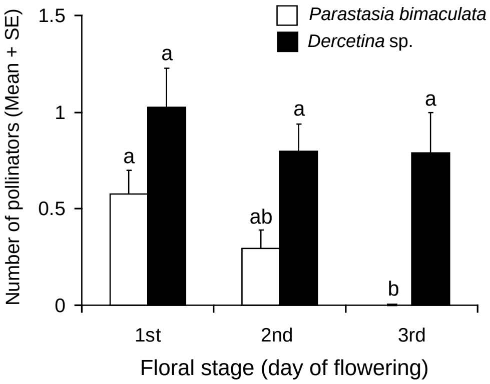


Fig. 1 Mean number (+ 1SE) of pollinators (*Parastasia bimaculata* and *Dercetina* sp.) arrivals on each floral stage inflorescence (the first day = 40 inflorescences, the second day = 40, the third day = 19). Different letters indicate significant differences (Sheffé test, P < 0.05). Open bars show *P. bimaculata*, and solid bars show *Dercetina* sp.

Table 1 Attractiveness of five main compounds in floral odors of *H. propinqua* and mixtures of them toward beetle pollinators (*Parastasia bimaculata* and *Dercetina* sp.).

Attractants [2]	Number of pollinator (mean ± SE)[1]	
	P. bimaculata	*Dercetina* sp.
α-Pinene (P)	0	0 *
2-Butanol (B)	0	0 *
2-Methyl-3-buten-2-ol (M)	0.13 ± 0 .13	0 *
2-Heptanol (H)	0	0 *
Veratrole (V)	0	1.71 ± 0.52
BP	0	0 *
BM	0	0.29 ± 0.18
BH	0	0.14 ± 0.14 *
BV	0.57 ± 0.30	2.29 ± 1.30
VM	0.29 ± 0.29	3.71 ± 1.43
VP	0	4.00 ± 1.09
VH	0	1.71 ± 0.47
BVP	0.29 ± 0.18	0.57 ± 0.20
BVM	0.14 ± 0.14	0.57 ± 0.20
BVH	0.86 ± 0.40	0.29 ± 0.18
BVPH	0.50 ± 0.31	1.20 ± 0.39
BVMH	0.13 ± 0.13	0.75 ± 0.25
BVPMH	0.60 ± 0.25	4.60 ± 1.44
Positive control (intact flowers	0.65 ± 0.15	2.70 ± 0.59

[1] Means in the same column with asterisk are significantly different between the positive control based on Dunnet test (P < 0.05). Each trial conducted on the separate day.

[2] Capital letters mean abbreviated names of five compounds. P: α-pinene, B: 2-butanol, M: 2-methyl-3-buten-2-ol, H: 2-heptanol, V: veratrole

Bioassays using the main components of the floral odors

The results of our bioassay are shown in Table 1. The positive controls (intact flowers) showed that *Dercetina* sp. arrived at inflorescences four times more frequently than *P. bimaculata*. No pollinators were attracted by empty Petri dishes (the negative controls). *Parastasia bimaculata* was attracted by 2-methyl-3-buten-2-ol (M), veratrole (1,2-dimethoxybenzene; V) plus M, and all mixtures containing 2-butanol (B) plus V thus the minimum components necessary for the attraction of *P. bimaculata* were M and BV. In contrast, *Dercetina* sp. was attracted by V, all mixtures containing V, and BM, which therefore appear to be the minimum components required for the attraction of *Dercetina* sp. Of these minimum components, BV and veratrole were the more efficient attractants for *P. bimaculata* and *Dercetina* sp., respectively.

Variations in the quantity of attractants in floral odors

Temporal variations in the quantity of the minimum components of attractants are shown in Table 2. On the first day of flowering, quantities both of BV and V increased significantly between the 0530 to 0630 period and the 0700 to 0800 period, then remained above the initial level (but not significantly) until the 10:00 to 11:00 period (Schéffe's test, $P < 0.05$). On the second day, the pattern was similar, but the quantity didn’t vary significantly among times for either attractant (Kruskal-Wallis test, $P > 0.05$). On the third day, the pattern was similar to that on the second day, but with a lower quantity of attractants, and quantities didn’t vary significantly among the five collection periods (Kruskal-Wallis test, $P > 0.05$). Variations in the

Table 2 Time variations in the quantity of attractants in floral odors of *H. propinqua* from 05:30 to 12:30 hours over the flowering periods (μg/ h / flower).

flowering day		Time				
		5:30-6:30	7:00-8:00	8:30-9:30	10:00-11:00	11:30-12:30
Attractants of *P. bimaculata*						
BV (2-butanol + Veratrole)						
1st	Median	**13.64** ^{a}	**97.54** ^{b}	**49.53** ^{ab}	**28.08** ^{ab}	**13.67** ^{a}
	Quatile	5.64 - 33.97	55.19 - 117.41	25.66 - 80.97	16.86 - 49.34	7.12 - 15.71
	Min - Max	2.38 - 57.92	52.26 - 194.08	11.88 - 141.39	13.54 - 80.62	6.33 - 63.25
2nd	Median	**41.32**	**77.76**	**41.76**	**38.69**	**11.7**
	Quatile	17.25 - 99.48	42.24 - 128.31	27.98 - 90.54	16.75 - 102.96	6.74 - 42.45
	Min - Max	2.38 - 456.27	29.23 - 172.37	22.89 - 121.78	5.28 - 106.98	2.73 - 85.00
3rd	Median	**11.89**	**12.36**	**14.53**	**15.51**	**3.66**
	Quatile	8.58 - 16.51	6.22 - 44.43	3.13 - 18.13	10.06 - 20.48	2.61 - 4.27
	Min - Max	2.69 - 23.93	7.89 - 163.61	0.81 - 19.85	1.74 - 23.82	1.21 - 4.78
Attractant compound of *Dercetina* sp.						
Veratrole						
1st	Median	**0.67** ^{a}	**16.29** ^{b}	**10.23** ^{ab}	**3.55** ^{ab}	**2.08** ^{a}
	Quatile	0.25 - 1.14	10.06 - 27.59	2.59 - 20.21	1.63 - 4.62	1.05 - 2.37
	Min - Max	0 - 6.06	5.50 - 29.55	1.78 - 34.95	1.49 - 5.34	0.46 - 5.40
2nd	Median	**6.46**	**18.02**	**8.06**	**4.44**	**3.9**
	Quatile	1.88 - 19.45	9.82 - 38.99	5.73 - 12.66	3.27 - 23.57	1.94 - 7.95
	Min - Max	0.29 - 205.95	2.51 - 53.19	2.10 - 21.93	2.86 - 25.50	0.91 - 18.25
3rd	Median	**1.66**	**7.02**	**4.05**	**1.91**	**1.88**
	Quatile	1.29 - 6.81	0.83 - 19.92	0.95 - 7.03	0.92 - 3.47	1.4 - 2.11
	Min - Max	0.21 - 15.73	0 - 141.25	0.11 - 9.28	0.14 - 3.87	0.13 - 2.63

Medians with different letter are significantly different among five collection times in each day (Sheffé test, $P < 0.05$).

quantity of attractants among floral stages are shown in Fig. 2. The quantity of BV decreased steadily over time, and the difference compared with the first day became significant on the third day (Steel-Dwass test, $P < 0.05$). The quantity of veratrole also decreased slightly from the first day to the third day, but the difference was not significant (Friedman's test, $P > 0.05$).

Discussion

In general, the pistillate phase (stigma receptivity) and the staminate phase (anther dehiscence) of protogynous Araceae inflorescences do not overlap, therefore obligate outcrossing seems to be the general rule (Mayo et al. 1997). Our results showed that *P. bimaculata* tended to arrive during the pistillate phase, when heat generation occurs; in contrast, *Dercetina* sp. arrived at inflorescences regardless of the floral stage (Fig. 1). To confirm the relationship between these behaviors and variations in the quantity of attractants, we examined the variations in quantities of BV and veratrole, and these were consistent with the frequency of arrivals of both insects during heat generation (Table 2) and among floral stages (Fig. 2). These results demonstrated that *P. bimaculata* is attracted by inflorescences that produce more BV when the volatilization of BV increases due to heat production by the spadices, whereas *Dercetina* sp. is attracted to even relatively small amounts of veratrole, and can thus arrive at inflorescences both during and after heat generation. Thus, *P. bimaculata* appears to have adapted to the protogynous *Homalomena* inflorescence. However, the number of *Dercetina* sp. arrivals was approximately four times that of *P. bimaculata* (Table 1, positive control), thus *H. propinqua* may be capable of attracting both specialized and general anthophagous pollinators to promote both the female and the male reproductive success.

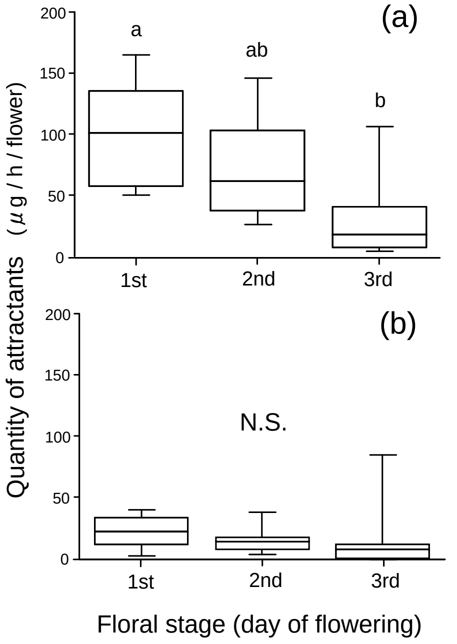


Fig. 2 Variations in the quantity of attractants among floral stages. Maximum and minimum values for each sample are shown at upper and lower ends of the vertical bars, respectively, and 75% and 25% points are given by the upper and lower ends of the box, respectively. The middle bar indicates median (each n = 10). Different letters indicate significant differences (Steel-Dwass test, $P < 0.05$). N.S. means no significant differences (Steel-Dwass test, $P > 0.05$). (a) BV (2-butanol + veratrole), (b) veratrole

References

Bernhardt P (2000) Convergent evolution and adaptive radiation of beetle-pollinated angiosperms. Plant Sys Evol 222:293-320

Gibernau M, Barabé D, Cerdan P, Dejean A (1999) Beetle pollination of *Philodendron solimoesense* (Araceae) in

French Guiana. Int J Plant Sci 160: 1135-1143

Gottsberger G, Silberbauer-Gottsberger I (1991) Olfactory and visual attraction of *Eriscelis emarginata* (Cyclocephalini, Dynastinae) to the inflorescence of *Philodendron selloum* (Araceae). Biotropica 23:23-28

Kato M (1996) Plant-pollinator interactions in the understory of a lowland mixed dipterocarp forest in Sarawak. Am J Bot 83:732-743

Kumano Y, Yamaoka R (2006) Synchronization between temporal variation in heat generation, floral scents and pollinator arrival in the beetle-pollinated tropical Araceae *Homalomena propinqua*. Plant Spec Biol 21:173-183

Mayo SJ, Bogner J, Boyce PC (1997) The genera of Araceae. Royal Botanical Gardens, Kew, UK.

Momose K, Yumoto T, Nagamitsu T, Kato M, Nagamasu H, Sakai S, Harrison RD, Itioka T, Hamid AA, Inoue T (1998) Pollination biology in a lowland dipterocarp forest in Sarawak, Malaysia. I. Characteristics of the plant-pollinator community in a lowland dipterocarp forest. Am J Bot 85:1477-1501

Young HJ. (1986) Beetle pollination of *Dieffenbachia longispatha* (Araceae). Am J Bot 73:931-944

Spatial Distribution of Two Bumble Bee Species in an Agro-Forestry Landscape

Atushi Ushimaru[1], Chikako Ishida[2], Shoko Sakai[2], Mitsue Shibata[3], Hiroshi Tanaka[4],
Takuo Nagaike[5], Kaoru Niiyama[4], Tohru Nakashizuka[6]
[1]Graduate school of Human Development and Environment,
Kobe University, 3-11 Tsurukabuto, Kobe 657-8501
[2]Center for Ecological Research, Kyoto University, Otsu 520-2113
[3]Forestry and Forest Products Research Institute, Morioka 020-0123
[4]Forestry and Forest Products Research Institute, Matsunosato, Tsukuba 305-8687
[5] Yamanashi Forest Research Institute, 2290-1 Saishoji, Masuho-cho, 400-0502
[6] Graduate School of Life Sciences, Tohoku University, Sendai 980-8577

Introduction

Bumble bees are important contributors to the pollination of temperate plants, including herbs, shrubs, and trees (Heinrich 1979; Yumoto 1987). They are important pollinators in both forests and open lands around artificially managed landscapes (Kato and Miura 1996; Walther-Hellwig and Frankl 2000; Osada et al. 2003; Yamazaki and Kato 2003), although little is known about their spatial distribution, i.e., habitat use, in these landscapes (Bhattacharya et al. 2003).

Forestry has changed natural forests into artificially structured forest landscapes. *Satoyama* forest is an east-Asian traditional forestry ecosystem in which many different human-managed landscape units, including both forest units (secondary forests, conifer plantations, and sometimes old-growth forests) and open-land units (cutover lands) are intermixed (Tabata 1997; Fukamachi et al. 2001). Different plant species compositions have been reported from different *satoyama* forest landscape units.

Two bumble bee species, the short-tongued *Bombus ardens ardens* Smith (*B. ardens*) and the long-tongued *B. diversus diversus* Smith (*B. diversus*), coexist and are dominant in *satoyama* forests (Kato and Miura 1996; Osada et al. 2003). *B. ardens* visits the simple-structured flowers of trees and shrubs (Yumoto 1987; Katayama 1996; Osada et al. 2003), whereas *B. diversus* generally prefers the complex floral structures of herbs, such as long corolla tubes (Washitani et al. 1994; Dohzono and Suzuki 2002). The local extinction of *B. diversus* because of habitat loss might reduce the sexual reproduction of plant species pollinated by this bee (Washitani et al. 1994). Eusocial *Bombus* species visit flowers of many plant taxa within a season. The diversity of woody species with shallow flowers is high in forests, whereas the diversity of herbaceous species with complex flowers is high both on the forest floor and in open lands. Thus, we predicted that within a given landscape, *B. ardens* would inhabit forests and *B. diversus* would inhabit both forests and open lands. Landscape units that lack flowering plant diversity, such as conifer plantation monocultures, would be avoided by both bumble bee species.

We used window traps to examine the spatial distribution (i.e., among several landscape units) of the two *Bombus* species in a *satoyama* forest. We also investigated the diversity of insect-pollinated plant species in five landscape units around the study area. We discuss the relationships between flower preference and the spatial distribution of the two *Bombus* species. We also address the management of landscape units for the conservation of the bumble bee pollination system in *satoyama* in which the traditional forest management system has been changing.

Materials and methods

Bumble bee species

Bombus ardens is widely distributed in Japan and Korea, except for Hokkaido and the Ryukyu Islands. B. *diversus* is endemic to the Japanese islands. The average colony life span of the two species differs widely; B. *ardens* lives from early April to early July, whereas B. *diversus* lives from late April to mid-October (Katayama 1996; Nakamura and Toquenaga 2002).

Study area and landscape units

In 2003, we investigated five landscape units around Ogawa Forest Reserve (OFR; 36°56’ N, 140°35’ E; 610–660 m elevation), which is located in the southern part of the Abukuma Mountains near Kitaibaragi City, Ibaraki Prefecture, Japan. We investigated five landscape units in and around OFR; these consisted of four forest units and one open-land unit:

- Old-growth deciduous forest (>100 years old): The dominant tree species were *Quercus crispula, Q. serrata*, *Fagus crenata,* and *F. japonica* (all Fagaceae), *Carpinus* spp. (Carpinaceae), and *Acer* spp. (Aceraceae) (Nakashizuka and Matsumoto 2002);
- Remnant forest (>100 years old): This consists of a narrow belt (30–50 m wide) of old-growth forest preserved as a boundary or firebreak. The dominant species were similar to those of the old-growth forest;
- Secondary deciduous forest (11–24 years old): The four secondary forests that we investigated were used for the production of mushroom logs and wood chips (Suzuki 2002). The dominant species were *Q. crispula*, *Q. serrata,* and *Carpinus laxiflora* (Suzuki 2002);
- Conifer plantation (25–50 years old): Two Japanese cedar (*Cryptomeria japonica*) and two hinoki cypress (*Chamaecyparis obtusa*) stands were investigated;
- Cutover lands (secondary forest 5–7 years after clear-cutting): *Rubus* spp., *Aralia elata,* and *Stephanandra incisa* were abundant, with a vegetation height of 2–4 m.

Bumble bee collection

We used window traps to collect bumble bees. The bumbles tended to strike against plastic transparent panes (45 cm high x 36 cm wide) and drop into a tray filled with 2 L of water and a small amount of detergent (Inari 2003). At the OFR old-growth forest and remnant forest, we set up four trap sites ≥ 200 m apart. We also set up trap sites in four secondary stands, four plantations, and three cutover lands (two sites in one large stand and two sites in two small stands). In all, we set up 20 study sites within an area of approximately 90 ha.

At each forest site, we set three traps at different heights to cover different strata: forest floor, 0.5 m;

shrub layer, ~3 m; and under-canopy layer, 8–20 m above the ground, depending on forest height. Two traps per site were set at a height of ~1.5 m in the cutover lands. A total of 56 traps were used. All traps were set at least 15 m inside each landscape unit. The window traps were set once a month from April to October, and the bumble bees were collected 6 days later.

For statistical analyses, we pooled data from the cutover lands and the pasture because these landscape units had similar floras (Fig. 3). We compared the total numbers of *B. ardens* and *B. diversus* individuals collected among the landscape units using a chi-square test. The expected number of individuals was calculated assuming a uniform distribution among the traps (we considered the number of traps per landscape unit). The distribution pattern between castes (queens and workers) was analyzed for *B. diversus*. We also used the chi-square test to examine the vertical distribution of the two species among forest units (primary, remnant, secondary, and plantation forests).

Diversity of insect-pollinated plant species in the landscape units

We investigated the insect-pollinated (entomophilus) plant diversity in the old-growth OFR stand, the remnant forest, two secondary forests (12 and 24 years old), two conifer plantations (both 32 years old), cutover lands (5 and 6 years old), and two pastures near OFR. We delineated one 10 x 100 m plot in each stand, except in one secondary forest in which the plot was 10 x 90 m. We inventoried all tree stems ≥ 5 cm in diameter at breast height and identified all shrubs ≥ 200 cm in height in each 5 x 5 m subplot. We also laid out 40 1 x 1 m quadrats inside each plot (36 quadrats in the young secondary forest plot) and inventoried all herbaceous species within each quadrat. We counted the number of entomophilus tree, shrub, and herb species for each plot and further subdivided herbs into early bloomers (April–June) and late bloomers (July–October). The number of species in each category was compared among the landscape units.

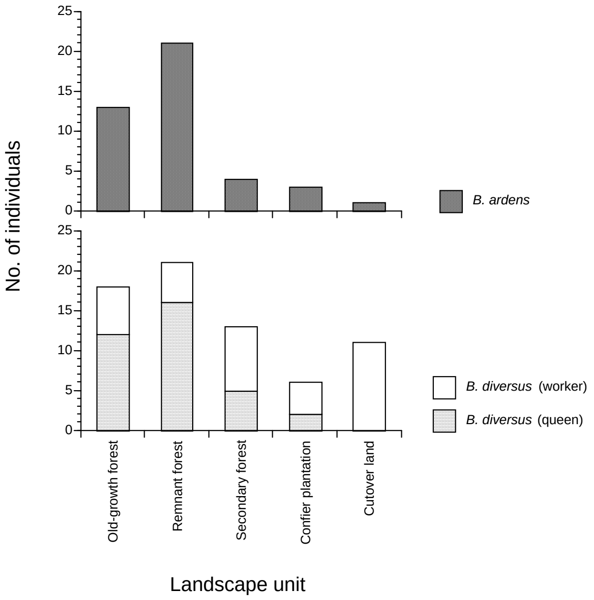


Fig. 1 Numbers of bumblebees collected in each landscape unit.
B. diversus individuals were divided into two groups: queens (shaded bars) and workers (open bars).

Results

Bumble bee collection using window traps

The total trappings for the year comprised 16 queens and 26 workers of *B. ardens*, 37 queens, 34 workers, and three males of *B. diversus*, and two queens and three workers of *B. hypocrita*. *B. ardens* queens were collected from April to June and workers from May to August. *B. diversus* queens were collected from April to June, whereas workers were collected from June to October (Fig. 1).

The number of *B. ardens* collected was significantly greater in old-growth and remnant forests than in other landscape units ($\chi^2 = 28.7$, d.f. = 4, $P < 0.001$; Fig. 1), the distribution pattern was not different between queens and workers. However, there was no significant difference in the total number of *B. diversus* collected among landscape units ($\chi^2 = 8.9$, d.f. = 5, $P > 0.05$; Fig. 1). *B. diversus* queens were caught more frequently in old-growth and remnant forests than in other units, but more workers were collected in cutover lands than in forest units (queens, $\chi^2 = 22.2$, d.f. = 5, $P < 0.001$; workers, $\chi^2 = 10.3$, d.f. = 5, $P < 0.05$; Fig. 1).

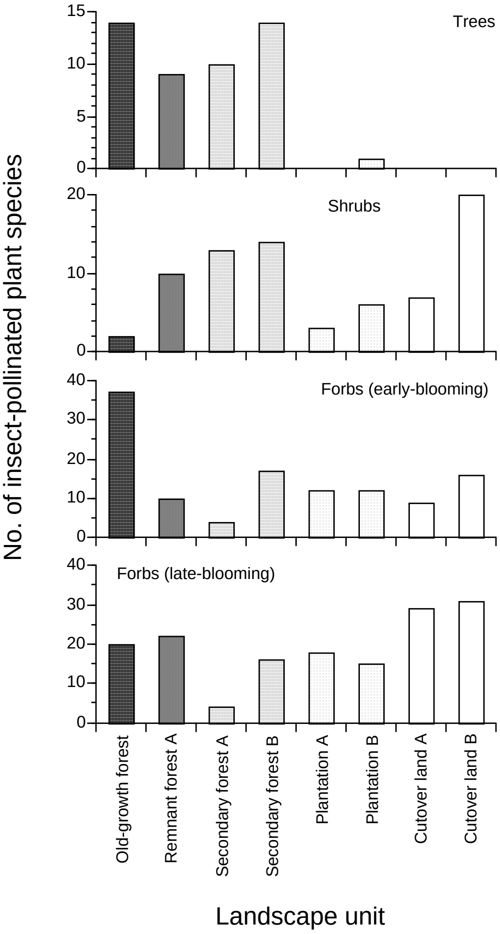


Fig. 2 Number of insect-pollinated plant species in five landscape types. Bumble bees were also trapped in the old-growth forest, remnant forest, secondary forest B, and cutover land A.

Diversity of insect-pollinated plant species in the landscape units

The diversity of entomophilus trees was high in old-growth, remnant, and secondary forests (Fig. 2). We rarely found entomophilus tree species in the canopy or subcanopy strata of plantations. Entomophilus shrub diversity was high in one cutover area and in secondary forests, but was relatively low in old-growth and remnant forests and plantations (Fig. 2). Among all the landscape units, the number of early-blooming herbs was largest in the old-growth forest (Fig. 2). Late-blooming herbs were more abundant in open-land units than in forest units (Fig. 2).

Discussion

Around OFR, most *B. ardens* queens and workers were caught in old-growth and remnant forests. We rarely collected *B. ardens* in secondary forests, conifer plantations, or cutover lands. We predicted that *B. ardens* inhabits forest vegetation in which woody species diversity is high. However, the diversity of woody species, i.e., trees and shrubs, was also high in secondary forests and cutover lands, respectively. Why was *B. ardens* not caught more frequently in these landscape units? The population dynamics of *B. ardens* are strongly affected by the abundance of tree flowers (Inari 2003). However, many trees in secondary forests and cutover lands were not large enough to flower. *B. ardens* may respond not only to woody diversity, but also to the abundance of flowers in trees.

As predicted, *B. diversus* was distributed in both forest and open-land units. Interestingly, we found a large difference in spatial distribution patterns between castes. Like *B. ardens* individuals, *B. diversus* queens were concentrated in old-growth and remnant forests, whereas the workers were more often trapped in open-land units. The rich diversity of early-blooming herbs on the floor of old-growth forests may attract the queens. However, why *B. diversus* queens were rarely caught in open-land units, where entomophilus herbaceous flower diversity is as high as that in old-growth, remnant, and secondary forests, is difficult to explain. Flowering individuals of spring ephemerals (e.g., *Erythronium japonicum*, *Corydalis lineariloba*, and *Viola* spp.) and shrubs (e.g., *Rhododendron obtusum* var. *kaempferi* and *Kerria japonica*) visited by *B. diversus* were not abundant in open-land units (A. Ushimaru personal observation). This scarcity of spring ephemeral flowers may be one reason that queens infrequently visit open-land units in the spring. In contrast, late-blooming herbs were found more frequently in open-land units than in forest units, suggesting that *B. diversus* queens forage on the flowers of forest herbs and shrubs until early summer, and workers spread into open lands to seek summer- and autumn-blooming herbs. Thus, the seasonal shift in the distribution of floral resources within the *satoyama* landscape results in inter-caste differences in spatial distribution.

We seldom collected bumble bees of either species in conifer plantations. During and after World War II, old-growth forests were widely replaced by conifer plantations (Nagaike et al. 2005). However, conifer plantations have not been extensively managed because of high costs. Infrequent logging and thinning has created a very dark forest floor, with consequent low understory plant diversity (Nagaike et al. 2005). Although forest-floor plants were not necessarily rare in plantations, we found very few flowering individuals because of low light conditions (A. Ushimaru, personal observation). This lack of flowers may account for the rarity of bumble bees.

Our results showed that old-growth stands are important habitat for both bumble bee species and for early-blooming insect-pollinated plants. In contrast, open lands play a significant role in the mutualism between *B. diversus* and late-blooming herbs. However, during the past decades, changes in official forest management policy have resulted in a rapid decrease in old-growth forests and cutover lands and an increase in conifer plantation monocultures (Fukamachi et al. 2001; Kato 2001; Inoue 2003; Nagaike et al. 2005). These landscape changes may have decreased the amount of habitat available for many plants and insects, including pollinators such as bumble bees (Tabata 1997; Kato 2001; Inoue 2003). The conservation of old-growth stands and the creation of new cutover lands by periodic logging are encouraged to conserve bumble bee–plant interactions.

Acknowledgements

We thank Naoki Inari for his suggestions on collecting bumble bees using window traps; Ryunosuke Tateno, Michiko Nakagawa, and Yuji Isagi for field assistance; and Takenari Inoue for valuable discussions on landscape use by bumble bees.

References

Bhattacharya M, Primack RB, Gerwein J (2003) Are roads and railroads barriers to bumblebee movement in a temperate suburban conservation area? Biol Consev 109: 37-45

Dohzono I, Suzuki K (2002) Bumblebee-pollination and temporal change of the calyx tube length in *Clematis stans* (Ranunculaceae). J Plant Res 115: 355-359

Fukamachi K, Oku H, Nakshizuka T (2001) The change of a satoyama landscape and its causality in Kamiseya, Kyoto Prefecture, Japan between 1970 and 1995. Landscape Ecol 16: 703-717

Heinrich B (1979) Bumblebee Economics. Harvard University Press, Cambridge, Massachusetts

Inari N (2003) Seasonal changes in vertical distribution of bumblebees and their floral resources within a cool-temperate deciduous forest in northern Japan. In: Toda M (ed) Reports on pollination network in a cool-temperate deciduous forest. Hokkaido University, Hokkaido, pp 27-41

Inoue T (2003) Butterfly fauna in and near the Ogawa Forest Reserve. Bull Forestry Forest Prod Res Inst 2: 237-246.

Katayama E (1996) Survivorship curves and longevity for workers of *Bombus ardens* Smith and *Bombus diversus* Smith (Hymenoptera, Apidae). Jap J Entomol 64: 111-121.

Kato M (2001) ‘Satoyama’ and biodiversity conservation: ‘Satoyama’ as important insect habitats. Glob Environ Res 5:135-149.

Kato M, Miura R (1996) Flowering phenology and anthophilous insect community at a threatened natural lowland marsh at Nakaikemi in Tsuruga, Japan. Contr Biol Lab Kyoto Univ 29:1-48

Nagaike T, Yoshida T, Miguchi H, Kamitani T, Nakashizuka T (2005) Rehabilitation for species enrichment in abandoned coppice forests in Japan. In: Stanturf JA, Madsen P (eds) Restoration of boreal and temperate forests. CRC Press, London, pp 371-381

Nakamura H, Toquenaga Y (2002) Estimating colony locations of bumble bees with moving average model. Ecol Res. 17: 39-48

Nakashizuka T, Matsumoto Y (Eds) (2002) Diversity and interaction in a temperate forest community: Ogawa Forest Reserve of Japan. Springer-Verlag, Tokyo.

Osada N, Sugiura S, Kawamura K, Cho M, Takeda H (2003) Community-level flowering phenology and fruit set: comparative study of 25 woody species in a secondary forest in Japan. Ecol Res 18: 711-723

Suzuki W (2002) Forest vegetation in and around Ogawa Forest Reserve in relation to human impact. In Nakashizuka, T., Matsumoto, Y. (Eds) Diversity and interaction in a temperate forest community: Ogawa Forest Reserve of Japan. Springer-Verlag, Tokyo, pp 27-40

Tabata H (ed) (1997) Satoyama and its conservation (in Japanese). Hoikusha, Osaka

Walther-Hellwig K, Frankl R (2000) Foraging habitats and foraging distances of bumblebees, *Bombus* spp. (Hym., Apidae), in an agricultural landscape. J Appl Entomol 124: 299-306

Washitani I, Osawa R, Namai H, Niwa M (1994) Patterns of female fertility in heterostylous *Primula sieboldii* under severe pollinator limitation. J Ecol 82: 571-579

Yamazaki K, Kato M (2003) Flowering phenology and anthophilous insect community in a grassland ecosystem at Mt. Yufu, western Japan. Contr Biol Lab Kyoto Univ 29: 255-318

Yumoto T (1987) Pollination systems of a warm temperate evergreen broad-leaved forest in Yakushima Island. Ecol Res 2: 133-145

Effects of Local Density and Forest Fragmentation on Reproductive and Regeneration Success of *Shorea laxa* (Dipterocarpaceae)

Yayoi Takeuchi[1], **Hiromitsu Samejima**[2], **Michiko Nakagawa**[3],
Bibian Diway[4], **Tohru Nakashizuka**[2]
[1]*Laboratory of Forest Biology, Graduate school of Agriculture, Kyoto University*
[2]*Graduate School of Life Sciences, Tohoku University*
[3]*Graduate school of Bioagricultural Sciences, Nagoya University*
[4]*Forest Research Center, Sarawak Forestry Corporation*

Introduction

Plant–animal interactions such as pollination, seed dispersal, and herbivory play pivotal roles in plant reproductive and regeneration processes (Ghazoul and McLeish 2001). The scale and intensity of these interactions are affected by the local density of host plants and their habitat area (Garcia and Chacoff 2007). Aggregation of host plants may enhance their pollination success, resulting in enhanced seed set, outcrossing, or escape from seed predation as a result of predator saturation. However, recent rapid deforestation, especially in the tropics, has severely reduced pollinator populations and species richness, decreasing the reproductive output of plants in remnant habitats and increasing inbreeding depression (Aizen and Feinsinger 1994; Young et al. 1996). Similarly, environmental change in the remnant forest also results in recruitment losses and rapid changes in species composition (Laurance et al. 2006).

In this study, we focused on the reproduction of *Shorea laxa* Slooten, one of the ecologically and commercially key dipterocarp species in the Southeast Asian tropical forest. We investigated whether reproductive success and seedling recruitment were affected by forest type (primary continuous forest versus fragmented forest, known locally as *pulau*) and local adult tree density in terms of (1) seed set, (2) seed predation by insects, (3) seed predation by vertebrates, (4) the selfing rate, and (5) seedling survival.

Materials and methods

Study site and species

We conducted our field study in and around Lambir Hills National Park (LHNP), Sarawak, Malaysia (4°2'N, 113°50'E; 150 to 200 m a.s.l.). We established a 80-ha study plots in LHNP, which contains continuous stands of primary forest. The fragmented forests resulted from traditional land use by local peoples and were located near LHNP (typically less than 1 km). These areas were originally primary forest, although some selective cutting of trees for house construction has occurred historically. One *pulau* was used for our study plot; it covered 5 ha, was located 200 m from the edge of the primary forest, was surrounded by secondary forests, and was located mainly in hilly terrain.

Shorea laxa (section Richetioides, Dipterocarpaceae) is commonly found in northeastern Borneo and becomes a canopy tree that reaches heights of 30 to 40 m. The mean density of mature stems in primary forest (diameter at breast height ≥ 30 cm) of *S. laxa* was 1.8 stems ha^{-1} (Takeuchi et al. 2005). The species flowers and produces seeds at irregular, supra-annual intervals. The fresh seed mass ± SD of *S. laxa* averages 18.3 ± 12.3 g (Takeuchi and Nakashizuka 2007). In LHNP, flowering occurred in early March 2005.

Census of flower and seed fate before seed dispersal, and seedling survival

Two seed traps (0.5 m^2 surface area) were placed beneath the crowns of 20 trees per plot in each type forest in early March 2005, just before flowering, and were monitored until late October 2005 to describe the patterns of seed fate and predation. Every week or two, we collected all seeds present in the seed traps. Seed traps were made of nylon cloth with a mesh size of 1 mm and were set 1 m above the ground. All collected seeds were counted, measured, and classified into four categories: sound, immature, attacked by insects, or attacked by vertebrates. Seeds that had holes on their surface or larvae or frass inside were categorized as having been attacked by insects. Seeds attacked by arboreal vertebrates were either broken into pieces or exhibited clear tooth marks. Seeds with insect damage rarely germinated; those that did were classified as sound (also see Nakagawa et al. 2005).

Seedling plots containing over 50 seedling individuals (ranging in size from 1×1 m to 1×5 m) were established beneath the canopy after seed dispersal in September 2005. Seedling survival was measured in 7 months later (i.e., in April 2006).

Environmental data

To characterize the light conditions at the forest floor, we measured the percent canopy openness using a digital camera with a fisheye lens (CoolPix 910, Nikon). Two or three images were taken beneath the canopy at each sampling site at ground level and at a 0.2-m height, and were analyzed using Gap Light Analyzer v2.0 (Frazer et al. 1999).

Under the canopy of each tree, we quantified the soil-water potential using the method of Deka et al. (1995). Soil samples were taken on the morning of 25 October 2006, after a relatively dry spell with no rain for 6 consecutive days. We extracted two 3-cm cores to a depth of 10cm beneath the canopy after removing the surface litter. Each soil sample was sealed in a plastic box for 8 days with filter paper (Whatman No. 42) placed in the soil. On 31 October 2006, the filter paper was weighed to within 1 mg after quickly removing soil with a small paintbrush, and the soil-water potential was calculated (Deka et al. 1995).

DNA analysis and estimation of selfing rate of seeds

Seed samples for estimating selfing rate were collected beneath the 14 focal trees in late August. Total DNA was extracted from the cotyledon (seeds) or cambium (adult trees) of each individual using the DNeasy Plant Mini Kit (Qiagen). Genotypes of DNA samples were scored using five to six pairs of microsatellite PCR primers that had been developed for *Dipterocarpus tempehes* Slooten (Isagi et al. 2002), *Shorea leprosula* Miq. (Lee et al. 2004), and *Shorea lumutensis* Sym. (Lee et al. 2006). For PCR, we used 10-µL reaction mixtures containing 1× PCR Buffer (Promega), 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 200 nM of each primer (one of each pair was fluorescently labeled), 1 ng of template DNA, and 0.25 to 0.50 units of *Taq* polymerase (Promega). PCR amplification was carried out for 5 min at 94°C, followed by 30 cycles of 30 s denaturing at 94°C, 30 s annealing at the optimized temperature, and 30 s extension at 72°C, with a final 3-min incubation at 72°C, using a GeneAmp^{TM} PCR System (Model 9700 and Model 2700; PE Applied Biosystems). The genotypes were determined using an ABI 3100 Genetic Analyzer and version 3.7 of the GeneScan^{TM} software (PE Applied Biosystems). To estimate whether the analyzed seed had been self-fertilized, we examined the genotype similarity between the seed and the presumed mother tree using Cervus 2.0 software (Marshall et al. 1998).

Nearest-neighbor index (PULAU)

We calculated a nearest-neighbor index (*NNI*) for each focal tree based on the proximity of conspecific trees:

$$NNI = \sum \left(\frac{1}{n_{ij}} \right)$$

where n_{ij} is the distance (m) between the focal tree*i* and the other conspecific tree*j* located within 200 m of the focal tree (Ghazoul and McLeish 2001). By summing the reciprocal of the distances, this index considers both the number of surrounding flowering trees and their distance, weighted more heavily for the nearest neighbors. Thus, a high index value indicates low isolation and the existence of many nearby neighbors.

Statistical analysis

Data were analyzed using the stepwise generalized linear mixed model (GLMM) techniques implemented in the R statistical software (http://www.r-project.org/) to test correlations between seed fate and *NNI*. For seedlings, we tested the effects of *NNI* and environmental variability across habitats (canopy openness and soil-water potential) on the seedling survival. The *NNI* values were ln-transformed, canopy openness values were arcsine-transformed, and water potential values (negative values, converted into positive values for analysis) were ln-transformed before the analysis.

Results

Effect of forest type

Large numbers of seeds fell before they had matured (97% or more, Table 1). The proportion of mature seeds was significantly higher in *pulau* than in primary forest (Table 1). The proportion of seeds >1 cm in diameter that were attacked by insects exceeded the proportion attacked by vertebrates in both forest types, but the difference was greater in *pulau*. Predation by vertebrates was significantly higher in the primary forest, but predation by insects did not differ significantly between forest types (Table 1). The selfing rate of mature seeds was significantly higher in primary forest than in *pulau* (Table 2).

Seedling survival did not differ significantly between primary forest and *pulau* (Table 3). *Pulau* had significantly more light (a greater canopy openness) and significantly less water (lower water potential) than the primary forest (Table 4). We found no interaction between light intensity and water potential (data not shown).

Table1 Number of focal tree, analyzed seeds and proportion of each seed fate.

Difference between forest type(primary vs. *pulau*) was tested by ANOVA.

Forest	No. of focal tree	Average no of total analyzed seeds±SD	Proportion of Mature seeds ±SD	Proportion of seed fate (＞1cm diameter) ±SD Insect	Vertebrates	Sound
Total	20	2058.2 ±923.8	0.027 ± 0.012	0.262 ± 0.094	0.147 ± 0.092	0.590 ± 0.137
Primary	15	2152.9 ±1011.2	0.026 ± 0.012	0.276 ± 0.101	0.183 ± 0.076	0.540 ± 0.115
Pulau	5	1774.0 ±583.0	0.030 ± 0.013	0.220 ± 0.061	0.040 ± 0.012	0.740 ± 0.070
p			<0.05	n. s.	<0.01	<0.01

Table 2 Number of focal tree and selfing rate in both forest type.
Difference between forest type (primary vs. *pulau*) was tested by ANOVA.All analyzed seeds were mature.

Forest	No. of focal tree	Average no of total analyzed mature	Selfing rate ±SD
Total	14	42.93 ± 17.26	0.080 ± 0.125
Primary	9	42.33 ± 5.34	0.094 ± 0.147
Pulau	5	44.00 ± 30.14	0.054 ± 0.080
p			<0.01

Table 3. Number of focal tree and survival rate (after 8 month) of seedlings in both forest types. Difference between forest type (primary vs. *pulau*) was tested by ANOVA.

Forest	No. of focal tree	Average no of total analyzed seedlings±	Survival rate ±SD
Total	11	50.0 ± 13.05	0.665 ± 0.173
Primary	6	58.0 ± 7.78	0.699 ± 0.184
Pulau	5	40.0 ± 11.55	0.623 ± 0.173
p			n. s.

Table 4. Environment diffarence between primary forest and *pulau*.

	Water Potential(kPa)±SD	Canopy Openness (%) ±SD
Primary	−0.01 ± 0.01	5.90 ± 0.54
Pulau	−0.28 ± 0.29	7.40 ± 0.40
p	<0.05	<0.05

Effect of local host density

Seed mature rates (the proportion of mature seeds) were negatively correlated with *NNI* in both forest types; the correlation was significant in primary forest and marginally significant in *pulau* (Table 5). The proportion of seeds predated by insects was not significantly correlated with *NNI* in either forest type, but the proportion of seeds predated by vertebrates was marginally positively correlated with *NNI* in the primary forest. We also found a significant negative correlation between the proportion of sound seeds and *NNI* in primary forest. The selfing rate of mature seeds also showed a significant and negative correlation with *NNI* in the primary forest, which suggests that isolation promotes self-fertilization. The correlation between *NNI* and the rates of sound seed production and selfing was not significant in *pulau*.

Seedling survival 8 months after seedling establishment in the primary forest was significantly affected by *NNI* and light intensity. On the other hand, seedling survival in *pulau* was negatively correlated with both light intensity (marginally significant) and water potential (significant), but we found no significant correlation with *NNI* (Table 6).

Table5 Results of stepwise GLMM for seed fate.

	Primary			Pulau		
		coefficient	p		coefficient	p
Mature rate	AIC:6981			AIC: 2215		
	Intercept	−4.652	<0.001	Intercept	−3.819	<0.001
	NNI_ln	−0.709	0.049	NNI_ln	−0.629	0.087
Predation by insects	AIC: 1551			AIC: 374.5		
	Intercept	−1.003	<0.001	Intercept	−1.204	<0.001
Predation by vertibrates	AIC: 1235			AIC: 120.4		
	Intercept	−0.500	0.380	Intercept	−3.136	<0.001
	NNI_ln	0.817	0.060			
Sound	AIC: 1809			AIC: 403.1		
	Intercept	−0.973	0.025	Intercept	0.997	<0.001
	NNI_ln	−0.878	0.007			
Selfing	AIC: 200			AIC: 59.67		
	Intercept	−9.285	0.001	Intercept	−2.637	0.030
	NNI_ln	−5.310	0.023	NNI_ln	5.700	0.220

Table6 Results of stepwise GLMM for seedling survival.

	Primary			Pulau		
		coefficient	p		coefficient	p
Seedling survival after 8 months	AIC: 317.8			AIC: 195.5		
	Intercept	3.2	0.051	Intercept	2.724	0.114
	NNI_ln	−8.081	<0.001	Openness_arcsin	−41.364	0.071
	Openness_arcsin	−193.243	<0.001	Water potential_ln	−0.523	0.002

Discussion

Effect of forest type on reproductive and recruitment success of **Shorea laxa**

We observed a significantly higher seed maturation rate in *pulau* than in primary forest. This suggests that pollination might function better in *pulau* than in primary forest. However, seed predation by vertebrates was significantly lower in *pulau* whereas predation by insects did not differ significantly between forests. These results can be explained if forest fragmentation has a stronger effect on populations of canopy vertebrates than on populations of insects. This is consistent with the results of Nakagawa et al. (2006), who also reported decreased rodent populations in *pulau* compared with primary forest. These results suggest that vertebrates may be more vulnerable than insects to forest fragmentation.

On the other hand, these differences did not lead to significant differences between forest types in terms of seedling survival 8 months after seedling establishment. However, the higher light intensity and decreased soil moisture in *pulau* compared with primary forest (Table 4) suggests that forest fragmentation may degrade the environment, even if these results are not apparent in the short term (i.e., our results reflect survival after less than 1 year). Seedling survival was affected by environmental conditions (Table 6). The long-term effect of the environmental changes that result from forest fragmentation may thus affect the survival pattern of seedlings.

In this study, we found that the difference between the *pulau* environment and the primary forest environment had no strong negative effects on the reproductive and recruitment success of *S. laxa*, at least in the short term (Tables 1 and 2). In other words, the *pulau* forest type, which represents a traditional form of forest reserve, may offer a similar function to that of primary forest for *S. laxa*.

Effect of local density on reproductive and recruitment success of **Shorea laxa**

Local host density affected the seed maturation rate and marginally the predation rate by vertebrates (Table 5). This suggests that aggregation of the study species had a negative effect on reproductive success in terms of both seed maturation and predation by vertebrates. It is not clear whether the decreased seed maturation rate results from seed abortion as a result of insect predation, which did not differ significantly among sites(Table 1), and/or inbreeding (selfing), which was significantly higher in primary forest(Table 2). The selfing rate of mature seeds decreased significantly with increasing local tree density, suggesting that tree isolation may increase the rate of pollination failure. The predation rate by vertebrates was marginally positively correlated with tree density in primary forest, suggesting that vertebrates respond to the local density of a food resource. On the other hand, we found that local host density did not affect insect predation in either forest type. This may result from differences in feeding behavior and range between insects and vertebrates.

Seedling survival was significantly correlated with *NNI* in primary forest. This indicates that predators, herbivores, and pathogens, which are a cause of seedling mortality in *S. laxa,* would exhibit density-dependent behavior.

In conclusion, our results show that *S. laxa* obtains advantages from low tree density in terms of seed maturation, avoidance of seed predation by vertebrates, and seedling recruitment. These results provide a possible explanation for why tropical trees tend to be sparsely distributed.

Acknowledgments

Field work was supported by B. Jinggan and DNA studies were assisted by M. Akedo. We greatly appreciate the kind support and organizational help from the Forest Department of Sarawak and the staff of the Forest Research Center of Sarawak. We also thank Dr. S. Sakai and members of the Laboratory of Forest Biology (Kyoto University) for their invaluable comments on the study. This study was financially supported by RIHN project P2-2 and a JSPS Research Fellowship for Young Scientists to Y. Takeuchi.

References

Aizen MA, Feinsinger P (1994) Habitat fragmentation, native insect pollinators, and feral honey-bees in Argentine Chaco Serrano. Ecol Appl 4:378-392

Deka RN, Wairiu M, Mullins PWC, Mullins E, Veenendaal EM, Townend J (1995) Use and accuracy of the filter-paper technique for measurement of soil matric potential. Eur J Soil Sci 46: 233–238.

Frazer GW, Canham CD, Lertzman KP (1999) Gap light analyzer (GLA). Simon Fraser University, Burnaby, British Columbia, and Institute of Ecosystem Studies, Millibrook, New York.

Garcia D, Chacoff NP (2007) Scale-dependent effects of habitat fragmentation on hawthorn pollination, frugivory, and seed predation. Conserv Biol 21:400-411

Ghazoul J, McLeish M (2001) Reproductive ecology of tropical forest trees in logged and fragmented habitats in Thailand and Costa Rica. Plant Ecol 153:335-345

Isagi Y, Kenta T, Nakashizuka T (2002) Microsatellite loci for a tropical emergent tree, *Dipterocarpus tempehes* V. Sl. (Dipterocarpaceae). Mol Ecol Notes 2:12-13

Laurance WF, Nascimento HEM, Laurance SG, Andrade A, Ribeiro JELS, Giraldo JP, Lovejoy TE, Condit R, Chave J, Harms KE, D'Angelo S (2006) Rapid decay of tree-community composition in Amazonian forest fragments. Proc Nat Acad Sci USA 103:19010-19014

Lee SL, Ng KKS, Saw LG, Lee CT, Muhammad N, Tani NTsumura Y, Koskela J (2006) Linking the gaps between conservation research and conservation management of rare dipterocarps: A case study of *Shorea lumutensis*. Biol Conserv 131:72-92

Lee SL, Tani N, Ng KKS, Tsumura Y (2004) Isolation and characterization of 20 microsatellite loci for an important tropical tree *Shorea leprosula* (Dipterocarpaceae) and their applicability to *S. parvifolia*. Mol Ecol Notes 4:222-225

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. Mol Ecol 7:639-655

Nakagawa M, Takeuchi Y, Kenta T, Nakashizuka T (2005) Predispersal seed predation by insects vs. vertebrates in six dipterocarp species in Sarawak, Malaysia. Biotropica 37:389-396

Takeuchi Y, Kenta T, Nakashizuka T (2005) Comparison of sapling demography of four dipterocarp species with different seed-dispersal strategies. For Ecol Manage 208:237-248

Takeuchi Y, Nakashizuka T (2007) Effect of distance and density on seed/seedling fate of two dipterocarp species. For Ecol Manage 247:167-174

Young A, Boyle T, Brown T (1996) The population genetic consequences of habitat fragmentation for plants. Trends Ecol Evol 11:413-418

Direct Genetic Analysis of Single Pollen Grains in Pollination Studies

Yu Matsuki, Yuji Isagi

Graduate School of Agriculture, Kyoto University

Introduction

Habitat fragmentation is a major threat to the biodiversity of forest ecosystems. During plant reproduction, habitat fragmentation disturbs gene flow and affects both the genetic structure and genetic diversity of progeny. Because insects are sensitive to habitat disturbances (Aizen and Feinsinger 2002), the pollination of insect-pollinated plants is often negatively affected by fragmentation (Young *et al*. 1996, Ghazoul *et al*. 1998, Benítez-Malvido and Martínez-Ramos 2003). In contrast, Dick *et al*. (2003) reported that long-distance pollen movement was enhanced in a fragmented site. These results reflect the complex and unpredictable responses of animal pollinators to fragmentation.

The genetic diversity and genetic structure of plant progeny are directly affected by both the quality and quantity of pollen grains that are transported to the stigma. Despite a wealth of data concerning the quantity of pollen transported to flowers (e.g., Herrera 1987, Mayfield *et al*. 2001), no studies have directly analyzed the genetic quality of transported pollen. The determination of genotypes from pollen grains carried by insects can reveal the genetic structure and diversity of transported pollen as well as the movement patterns of flower-visiting insects. This method may also provide a novel approach for evaluating human impacts on forest ecosystems.

We developed a new method of multiple microsatellite genotyping to analyze the pollination of insect-pollinated plants. We confirmed the reliability of the method by analyzing the genotypes of pollen grains transported by flower-visiting insects.

Materials and Methods

Plant species

Magnolia obovata Thunb. (Magnoliaceae) is a large (up to 20–30 m in height), common deciduous tree species native to temperate forests in Japan. The standing density of adult trees is relatively low, with only a small number of trees per hectare (Isagi *et al*. 2004). Flowers of this species are hermaphroditic and protogynous. The primary pollinators of *M. obovata* are beetles (Kikuzawa and Mizui 1990, Isagi *et al*. 2004), which crawl through the flowers for long periods of time (Thien 1974). Bees, hover flies, and thrips also visit the flowers of *M. obovata* (Tanaka and Yahara 1998).

Study site

A field survey was conducted at the Ogawa Forest Reserve, Ibaraki Prefecture, central Japan (36˚56’ N, 140˚35’ E). The average annual mean air temperature and precipitation over 10 years (1986–1995) at a meteorological station in Ogawa (36˚54’ N, 140˚35’ E) were 10.7°C and 1910 mm, respectively (Mizoguchi *et al*. 2002). The area is covered by a deciduous broad-leaved forest, and the dominant woody species in the canopy are *Quercus serrata*, *Fagus japonica*, and *F. crenata*. Research in the reserve has included intensive studies of the structure and dynamics of the plant community (Nakashizuka *et al.* 1992) and the population dynamics of *Carpinus* (Shibata and Nakashizuka 1995), *Acer* (Tanaka 1995), and *Cornus* (Masaki *et al.* 1994) species. Several types of land use (e.g., conifer plantations, paddy fields, pastures) surround the forest reserve.

Sampling

During the flowering period of *M. obovata* in 2004 and 2005, fresh stamens and flower-visiting insects were collected from six adults of *M. obovata*. Each insect was collected immediately after visitation. Samples were stored at –30˚C prior to DNA analysis.

DNA extraction from a single pollen grain

For DNA extraction from a single pollen grain, we modified the extraction method described by Suyama *et al.* (1996). Extraction buffer (1 μL) containing 0.01% SDS, 0.1 μg/μL proteinase K (TaKaRa, Tokyo. Japan), 1× PCR buffer (containing 1.5 mM $MgCl_2$) of Ampli*Taq* Gold (Applied Biosystems, Foster City, CA) was placed into a 0.2 mL PCR tube. Under a stereomicroscope, a single pollen grain was removed from the stamen surface using a plastic pipette tip (for manipulating 0.5–10 μL of liquid) that had been stretched and cut to obtain a sharp end. One pollen grain was then placed into the buffer and crushed using a sterile plastic pipette tip (for manipulating 20–200 μL of liquid). The reaction buffer was incubated at 37˚C for 60 min and then at 95˚C for 10 min.

Determination of multiple microsatellite genotypes

Because a single pollen grain contains only a small amount of haploid nuclear material, it is impossible to divide template DNA into multiple reaction tubes to amplify multiple microsatellite loci. Therefore, to obtain multiple microsatellite genotypes from a single pollen grain, we had to either conduct whole genome amplification before microsatellite genotyping or genotype several loci simultaneously in a single reaction tube. We tested both methods for obtaining genotypes of multiple microsatellite loci from single pollen grains.

LL-DOP PCR for whole genome amplification

LL (long products from low DNA quantities)-DOP (degenerate oligonucleotide-primed) PCR (Kittler *et al.* 2002) is one method for whole genome amplification from a small amount of DNA. This method can generate long products from a small amount of template DNA with high fidelity (Kittler *et al.* 2002). The entire genomes of 16 pollen grains were amplified separately using the Expand High Fidelity PCR System (Roche, Basel, Switzerland) following the manufacturer's protocol. Using amplified DNA from a single pollen grain as a template, 11 microsatellite loci developed by Isagi *et al.* (1999) were amplified separately in 11 reaction tubes.

Multiplex PCR

The multiplex PCR method (Chamberlain *et al.* 1988) can amplify multiple loci simultaneously in a single reaction tube using a small quantity of DNA. Genotypes of nine microsatellite loci of 20 pollen grains were scored using a Multiplex PCR kit (Qiagen, Hilden, Germany) following the manufacturer's protocol.

Genotypes were determined using an ABI PRISM 3100 Genetic Analyzer, GENESCAN^{TM} analysis software version 3.7, and GENOTYPER^{TM} analysis software version 2.0 (all from Applied Biosystems). The genotypes of pollen grains were compared to those of the pollen parent from which the stamen was sampled.

Genotyping of pollen grains adhering to flower-visiting insects

Among the observed flower-visiting insects, bumblebees (Apidae, *Bombus*), flower beetles (Scarabaeidae, Cetoniinae), and small Coleoptera (Ligriidae, *Arthromacra*) were used to confirm the effectiveness of our analysis method. We removed 47–48 pollen grains (143 grains total) from the surfaces of a bumblebee (*Bombus diversus*), a flower beetle (*Protaetia cataphracta*), and a small beetle (*Arthromacra sumptuosa*). The genotypes of nine microsatellite loci of each pollen grain were determined using the multiplex PCR method. The pollen samples for which genotypes were determined for more than five microsatellite loci were used for analysis. The percentage of self-pollen (i.e., pollen transported within a tree) was calculated for each insect. The genetic diversity of transported pollen grains was expressed in terms of gene diversity (Nei 1973).

Results

LL-DOP PCR

Among 176 combinations of 11 loci and 16 pollen grains, 71 genotypes (40.0%) were successfully determined. The proportion of successful genotyping differed among the microsatellite loci (Fig. 1), ranging from 0.06 (locus: *M15D5*) to 0.94 (locus: *M6D1* and *M6D8*).

Multiplex PCR

Among 180 combinations of nine loci and 20 pollen grains, 163 genotypes (approx. 90.6%) were successfully determined, and all of the amplified alleles were consistent with those of the pollen parent from which the stamen was collected (Fig. 2). Although the proportion of successful genotyping differed among the microsatellite loci (Fig. 1), the variances were smaller than those of the LL-DOP PCR method.

Genotyping of pollen grains adhering to flower-visiting insects

In the analysis of pollen grains that adhered to flower-visiting insects, we were able to determine genotypes for more than five microsatellite loci of 134 pollen grain samples. The percentage of self-pollen in the pollen load was 93.6% on bumblebees, 12.5% on flower beetles, and 95.7% on small beetles. The gene diversity of pollen grains from bumblebees, flower beetles, and small beetles was 0.36, 0.79, and 0.47, respectively.

Discussion

Comparison of analysis methods

We compared two methods of PCR amplification for determining the genotypes of multiple microsatellite loci from a single pollen grain. The proportions of successful genotyping in the LL-DOP PCR method were lower than those in the multiplex PCR method. In addition, the variance in the success rate of each locus was high in the LL-DOP PCR method. Whole genome amplification methods do not always amplify the entire genome, and a portion of genome may often be lost (Wells *et al.* 1999). Therefore, it may be difficult to amplify multiple regions that include microsatellite loci from one copy of the haploid nuclear genome of a single pollen grain. Thus, we determined that the LL-DOP PCR method was unsuitable for the genetic analysis of pollen grains.

In contrast, the multiplex PCR method successfully genotyped a single pollen grain with high fidelity. Failed genotyping was unevenly distributed among individual loci, suggesting that these failures resulted from traits of each locus or the PCR primers. The multiplex PCR method is suitable for analyzing pollen grains because it amplified pollen DNA with high success rates and fidelity. In addition, this method can be completed in less time and at a lower cost than the LL-DOP PCR method.

Genotyping of pollen grains adhering to flower-visiting insects

Among the 143 pollen grains analyzed, the genotypes of 134 pollen grains (93.7%) were determined for more than five microsatellite loci. This result indicates that the method is practical and effective for detailed studies on pollination. We found large differences in the percentage of self-pollen and gene diversity among insect species, suggesting that the visitation behavior of insects varies with species. Differences in visitation behavior can result in varying effects on the reproduction and fitness of pollinated *M. obovata*. The present method of genetically analyzing a single pollen grain may facilitate more detailed pollination studies, including research on pollinator efficiency and human impacts on pollination systems.

References

Aizen MA, Feinsinger P (2002) Bees not to be? Responses of insect pollinator faunas and flower pollination to habitat fragmentation. In: Bradshaw GA and Mooney HA (eds) How landscapes change: human disturbance and ecosystem fragmentation in the Americas. Springer, Berlin, pp 111–129

Benítez-Malvido J, Martínez-Ramos AM (2003) Impact of forest fragmentation on understory plant species richness in Amazonia. Conserv Biol 17: 389–400

Chamberlain JS, Gibbs RA, Ranier JE, Nguyen PN, Caskey CT (1988) Deletion screening of the Duchenne muscular dystrophy locus via multiplex DNA amplification. Nucleic Acids Res 16: 11141–11156

Dick CW, Etchelecu G, Austerlitz F (2003) Pollen dispersal of tropical trees (*Dinizia excelsa*: Fabaceae) by native insects and African honeybees in pristine and fragmented Amazonian rainforests. Mol Ecol 12: 753–764

Ghazoul J, Liston KA, Boyle TJ (1998) Disturbance-induced density-dependant seed set in *Shorea siamensis* (Dipterocarpaceae), a tropical forest tree. J Ecol 86: 462–473

Herrera CM (1987) Components of pollinator ‘quality’: comparative analysis of a diverse insect assemblage. Oikos 50: 79–90

Isagi Y, Kanazashi T, Suzuki W, Tanaka H, Abe T (1999) Polymorphic DNA markers for *Magnolia obovata* Thunb. and their utility in related species. Mol Ecol 8: 698–700

Isagi Y, Kanazashi T, Suzuki W, Tanaka H, Abe T (2004) Highly variable pollination patterns in *Magnolia obovata* revealed by microsatellite paternity analysis. Int J Plant Sci 165: 1047–1053

Kikuzawa K, Mizui N (1990) Flowering and fruiting phenology of *Magnolia hypoleuca*. Plant Species Biol 5: 255–261

Kittler R, Stoneking M, Kayser M (2002) A whole genome amplification method to generate long fragments from low quantities of genomic DNA. Anal Biochem 300: 237–244

Masaki T, Kominami Y, Nakashizuka T (1994) Spatial and seasonal patterns of seed dissemination of *Cornus controversa* in a temperate forest. Ecology 75: 1903–1910

Matsuki Y, Isagi Y, Suyama Y (2007) The determination of multiple microsatellite genotypes and DNA sequences from a single pollen grain. Mol Ecol Notes 7: 194–198

Mayfield MM, Waser NM, Price MV (2001) Exploring the ‘Most Effective Pollinator Principle’ with complex flowers: bumblebees and *Ipomopsis aggregata*. Ann Bot 88: 591–596

Mizoguchi Y, Morishita T, Ohtani Y (2002) Climate in Ogawa Forest Reserve. In: Nakashizuka T, Matsumoto Y (eds) Diversity and interaction in a temperate forest community. Springer, Tokyo, pp 11–18

Nakashizuka T, Iida S, Tanaka H, Shibata M, Abe S, Masaki T, Niiyama K (1992) Community dynamics of Ogawa Forest Reserve, a species-rich deciduous forest, central Japan. Vegetatio 103: 105–112

Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA 70: 3321–3323

Shibata M, Nakashizuka T (1995) Seed and seedling demography of four co-occurring *Carpinus* species in a temperate deciduous forest. Ecology 76: 1099–1108

Suyama Y, Kawamuro K, Kinoshita I, Yoshimura K, Tsumura Y, Takahara H (1996) DNA sequence from a fossil pollen of *Abies* spp. from Pleistocene peat. Genes Genet Syst 71: 145–149

Tanaka H (1995) Seed demography of three co-occurring *Acer* species in a Japanese temperate deciduous forest. J Veg Sci 6: 887–896

Tanaka H, Yahara T (1988) The pollination of *Magnolia obovata*. In: Kawano S (eds) The world of plants, vol. 2. Kenkyusha, Tokyo, p 37

Thien LB (1974) Floral biology of *Magnolia*. Am J Bot 61: 1037–1045

Wells D, Sherlock JK, Handyside AH, Delhanty JDA (1999) Detailed chromosomal and molecular genetic analysis of single cells by whole genome amplification and comparative genomic hybridization. Nucleic Acids Res 27: 1214–1218

Young A, Boyle T, Brown T (1996) The population genetic consequences of habitat fragmentation for plants. Trends Ecol Evol 11: 413–418

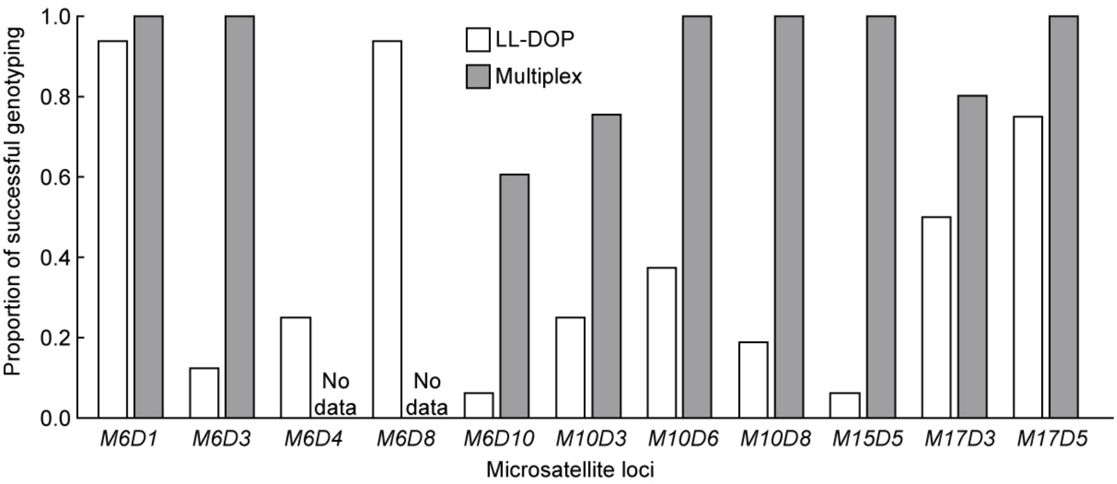


Fig. 1 The proportion of successful genotyping of microsatellite loci using the LL-DOP PCR and multiplex PCR methods. We examined 16 and 20 pollen grains using the LL-DOP PCR and multiplex PCR methods, respectively. Genotype determination was considered successful when the allele corresponding to the pollen parent was obtained. 1

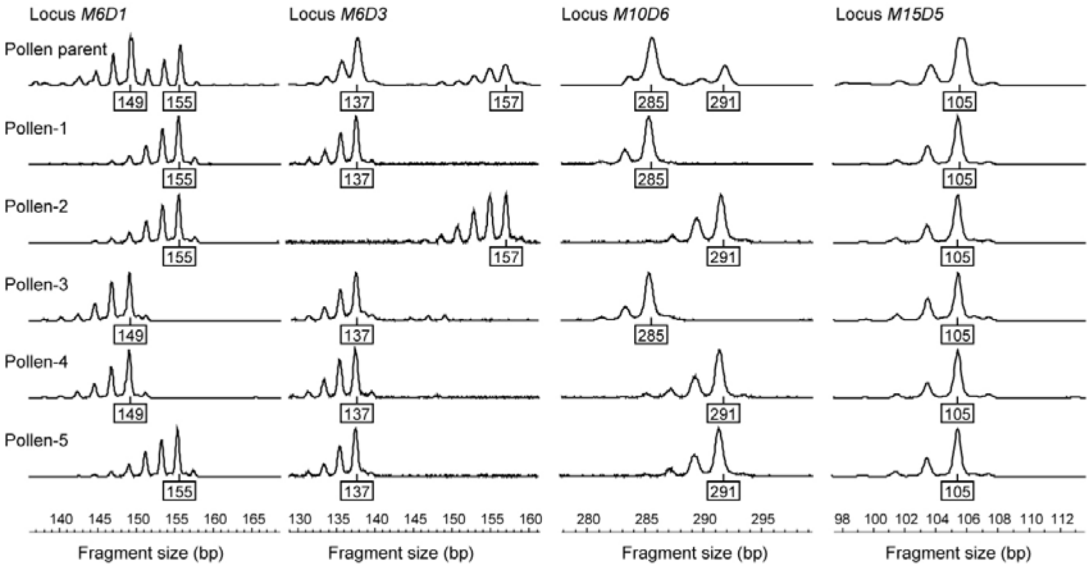


Fig. 2 Electropherograms of alleles at four microsatellite loci (*M6D1*, *M6D3*, *M10D6*, *M15D5*) of pollen parents and pollen grains amplified using the multiplex PCR method (modified from Matsuki *et al*. 2007).

The Effects of Forest Fragmentation on Population Structure and Reproductive Output in Populations of *Magnolia obovata*

Ryunosuke Tateno[1], Yu Matsuki[2], Yuji Isagi[2], Mitsue Shibata[3]
[1] *Faculty of Agriculture, Kagoshima University*
[2] *Graduate School of Agriculture, Kyoto University*
[3] *Forestry and Forest Products Research Institute*

Introduction

Forest fragmentation caused by anthropogenic activity is a threat to the continuation of local populations and communities throughout the world, because it causes a decrease in the number of conspecific reproductive individuals, which can result in decreased reproductive output and, eventually, increased risk of extinction. Forest fragmentation also changes environmental conditions via edge effects, which can positively or negatively affect seedling establishment, growth, and reproductive output of trees. Animal-pollinated plant reproductive output can be influenced through changes in local animal assemblages, caused by changes in behavior and flight patterns, in response to fragmentation (Aizen and Feinsinger 1994; Didham 1996). Thus, forest fragmentation can directly and indirectly affect reproductive output; however, few studies have investigated the effect of forest fragmentation on population structure and reproductive output of tree species.

Many studies have examined reproductive output of herbaceous species and shrubs. However, few studies of reproduction in tall tree species have addressed reproductive processes by marking or sampling reproductive twigs, but it is laborious to estimate reproductive output at the individual level. In this study, we addressed whole plant reproductive output, including flowering, fertilization, and seed set.

The study species, *Magnolia obovata*, is ubiquitous in the cool temperate forests of Japan, but at a relatively low density. It is not clear how such sparse tree populations are maintained. This species has a large reproductive organ, which makes it easy to evaluate the reproductive output at the whole plant level throughout the reproductive process.

The aim of this study was to evaluate the effect of forest fragmentation on the population structure and reproductive output of *M. obovata*. We compared tree density, size distribution patterns, reproductive characteristics (flower number, fruit set, and seed number per fruit) at two nearby sites, one conserved and one fragmented, in the southern part of the Abukuma Mountains, Ibaraki Prefecture, central Japan, over a three-year period.

Materials and Methods

Study Site

The study was carried out in the Ogawa Forest Reserve and its surrounding area, in the southern part of the Abukuma Mountains, Ibaraki Prefecture, central Japan (36°56´N, 140°35´E; altitude 610–660 m above sea level). Mean annual air temperature and precipitation at a meteorological station in Ogawa (36°54´N, 140°35´E) during the study period were 10.7°C and 1910 mm, respectively (Moriguchi et al. 2002). The study area is covered by deciduous broadleaf forest, and the dominant woody species in the canopy are *Quercus serrata*, *Fagus japonica*, and *F. crenata* (Nakashizuka et al. 1992; Masaki et al. 1994).

The study site was about 2 km × 3 km, and included lands with various uses, such as conserved natural forest (98 ha), fragmented natural forest (29 ha), secondary forest, coniferous plantations, farmland, and pasture. The study was conducted in the conserved natural forest and the fragmented natural forest (Fig. 1).

The fragmented forest is shaped like a fish bone, and is located about 500 m from the conserved forest (Fig. 1). According to forest management notes and interviews with local people, forest fragmentation was caused by clear cutting of broadleaf forest and planting of coniferous trees in the 1970s. The vegetation of the conserved forest is thought to be similar to that of the fragmented forest. Secondary forests and coniferous plantations surround the conserved and fragmented forests. The secondary forests include several deciduous broadleaf species, such as *Quercus serrata*, and are used for charcoal production by the local people.

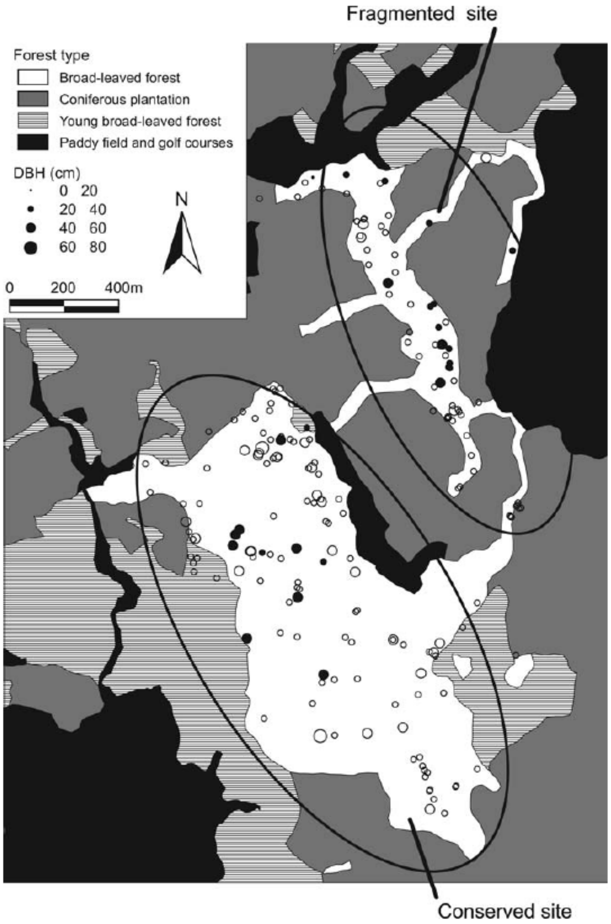


Fig. 1 The sizes and locations of reproductive *Magnolia obovata* trees in the conserved and fragmented forest sites. Filled circles: Adult trees from which fruits were collected (Isagi et al. 2007).

Study Species

The flower of *M. obovata* is one of the largest in the region, protogynous and without nectaries. The main pollinators of the flowers are beetles, bumble bees, and halictid bees (Tanaka and Yahara 1988; Tateno et al. personal observation). Although individual flowers last for 3–4 days, a tree flowers for up to 40 days, from late May to early July, in this study area (Tateno et al. unpublished data). Seeds reach maturity in mid-September at the study site and are dispersed by birds.

Field Study and Sampling

Reproductive *M. obovata* trees were identified and the location of each tree was determined with a portable GPS (eTrex Summit, Garmin, Kansas, USA). We measured diameter at breast height (DBH) of all trees and checked the flowering status of each tree.

Eight individuals (22.1–56.4 cm DBH) per forest type were selected for counting flower number and fruit number from the conserved and the fragmented forests. We accessed the canopy by ladder or climbing ropes, or both, and sketched almost all of the flowers or flower buds on a branch during the flowering period. We checked for fruit set in late September.

We selected fourteen individuals (18.0–56.6 cm DBH) for fruit sampling from the conserved forest and the fragmented forest. Five or more mature fruits were collected from each sampled tree; however, in non-fruiting years, we were unable to collect five fruits per individual. The numbers of ovules and mature seeds in each fruit were counted.

Results and Discussion

The density of trees was 1.3 trees/ha in the conserved forest and 1.9 trees/ha in the fragmented forest. Although there were no differences in size distribution patterns of large trees between the conserved and the fragmented forests, the number of small individuals (<40 cm DBH) was higher in the fragmented forest than in the conserved forest (Fig. 2). These small trees can establish in forest edges and roadsides at the time of clear-cutting of surrounding forests. According to the distribution map of individuals, some patches containing small individuals were located along the forest edge and roadside in the fragmented forest (Fig. 1). The percentage of reproductive trees increased with size, and all trees with DBH greater than 30 cm were reproductive (Fig. 2). These results suggest that, in the near future, the density of reproductive individuals should increase much more in the fragmented forest than in the conserved forest.

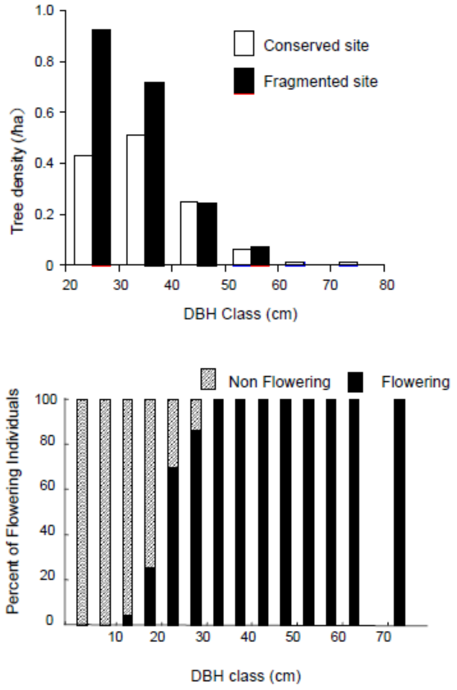


Fig. 2 Size distributions of *Magnolia obovata* trees and the percentage of flowering individuals.

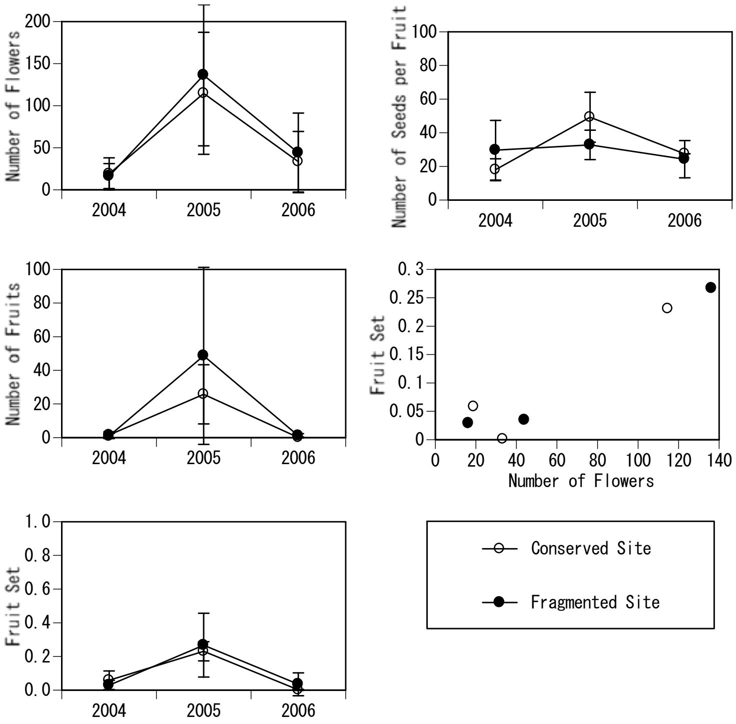


Fig. 3 Interannual changes in reproductive properties of *Magnolia obovata* in the conserved and fragmented forests.

The trees flowered and produced fruit every second year, and were synchronized between the conserved forest and the fragmented forest (Fig. 3). There was a clear positive correlation between flower number and fruit set (Fig. 3), suggesting that increased flower number results in increased fruit set in *M. obovata* of this site. Kikuzawa and Mizui (1990) showed significantly increased fruit set by self-pollination and concluded that populations of *M. obovata* were pollinator-limited in a deciduous broadleaf forest in northern Japan. In contrast to fruit set, seed number per fruit did not show clear inter-annual variation. Seed number per fruit varied widely among fruits. Isagi et al. (2004) found that outcrossing rate and number of pollen donors varied widely among fruits. Pollination is highly variable at the level of fruits and individuals, possibly because of high variability in pollinator behavior. Seed number per fruit may be affected by several other processes, such as seed predation and abortion.

Although the average reproductive output did not differ significantly between the conserved and fragmented forests, the responses of reproductive processes to the local density of reproductive individuals differed between the two sites. Isagi et al. (2007) reported that the fertilization of ovules and outcrossing were more dependent on density of reproductive individuals in the fragmented forest than in the conserved forest. Similarly, fruit set in this study was dependent on density of reproductive individuals in the fragmented forest; however, this relationship was not significant in the conserved forest (Tateno et al. unpublished data). These results suggest that compensatory mechanisms for the low density may operate in the conserved forest more effectively than in the fragmented forest. We speculate that foraging area of pollinators may be wider in the conserved forest because it may be easier for them to forage in the canopy of deciduous trees. In the fragmented forest, the canopy of deciduous trees was interspersed with coniferous plantations, farmland, and pasture, and pollinators may concentrate near trees growing in the congested areas of conspecific trees. A better understanding of pollinator foraging behavior will be important for predicting the effect of fragmentation on population dynamics of this species.

Forest fragmentation may favor *M. obovata* populations because of the increasing number of safe sites for seedling establishment, growth, and future reproduction. Currently, the reproductive outputs of the two forest sites were not significantly different, but in the future the density of reproductive trees will increase in the fragmented forest, which will weaken pollen limitation and increase reproductive output. However, the fragmented populations may suffer negative genetic effects, such as inbreeding depression, increased susceptibility to diseases and pests, fixation of deleterious alleles, and loss of self-incompatibility alleles. Future studies are required to predict the effects of forest fragmentation, both the ecological and genetic consequences, on *M. obovata* population dynamics.

Acknowledgments

This study was financially supported by the Research Institute for Humanity and Nature (Project No. 2-2) and the Forestry and Forest Products Research Institute.

References

Aizen MA, Feinsinger P (1994) Forest fragmentation, pollination, and plant reproduction in a Chaco dry forest, Argentina. Ecology 75:330–351

Didham RK, Ghazoul J, Stork NE & Davis AJ . (1996). Insects in fragmented forests: a functional approach. Trends in Ecology and Evolution 11:255-260

Isagi Y., Kanazashi T., Suzuki W., Tanaka H., Abe T. (2004) Highly Variable Pollination Patterns in *Magnolia obovata* Revealed by Microsatellite Paternity Analysis *International Journal of Plant Sciences* 165:1047–1053

Isagi Y., Tateno R., Matsuki Y., Hirao A., Watanabe S. Shibata M.(2007) Genetic and reproductive consequences of forest fragmentation for populations of *Magnolia obovata*. Ecological Research 22:382-389

Kikuzawa K, Mizui N (1990) Flowering and fruiting phenology of *Magnolia hypoleuca.* Plant Species Biol 5:255–261

Masaki T, Kominami Y, Nakashizuka T (1994) Spatial and seasonal patterns of seed dissemination of *Cornus controversa* in a temperate forest. Ecology 75:1903–1910

Moriguchi Y, Morishita T, Ohtani Y (2002) Climate in Ogawa Forest Reserve. In: Makashizuka T, Matsumoto Y (eds) Diversity and interaction in a temperate forest community. Springer, Tokyo, pp 11–18

Nakashizuka T, Iida S, Tanaka H, Shibata M, Abe S, Masaki T, Niiyama K (1992) Community dynamics of Ogawa Forest Reserve, a species-rich deciduous forest, central Japan. Vegetatio 103:105–112

Tanaka H, Yahara T (1988) The pollination of *Magnolia obovata*. In: Kawano S (eds) The world of plants, vol. 2. Kenkyusya, Tokyo, p 37

Analysis of Mating Patterns and Spatial Genetic Structure in *Acer mono* Using Microsatellite Genetic Markers in Conserved and Fragmented Forests

Satoshi Kikuchi[1], Mitsue Shibata[2], Hiroshi Yoshimaru[1], Hiroshi Tanaka[1], Kaoru Niiyama[1]

[1]Department of Forest Genetics, Forestry and Forest Products Research Institute,
Matsunosato, Tsukuba, Ibaraki 305-8687, Japan
[2]Tohoku Research Center, Forestry and Forest Products Research Institute
Morioka, Iwate 020-0123, Japan

Introduction

Acer mono Maxim. (Aceraceae) is a deciduous canopy tree widely distributed in eastern Asia, and one of the major components in deciduous cool-temperate forests of Japan. The genus *Acer* exhibits great variability in its reproductive systems, including monoecy, androdioecy, and dioecy, and *A. mono* shows heterodichogamy, as do most *Acer* species (de Jong 1994). Heterodichogamy, by definition, refers to a breeding system that involves two types of bisexual individuals (protandrous and protogynous individuals) in a population and has been reported from 11 families and 17 genera of flowering plants, including *Grayia* (Chenopodiaceae), *Juglans* (Juglandaceae), and *Acer* (Aceraceae). In heterodichogamous taxa in genus Acer, male individuals occasionally occur at a low frequency (heterodichogamous androdioecy, Gleiser & Verdú 2005).

Several previous studies addressing sex expression in these taxa have revealed the reciprocal and synchronous nature of two flowering types, and heterodichogamy is considered an effective mechanism to avoid selfing and to ensure outcrossing by promoting between-type mating (Gleeson 1982, Pendleton *et al*. 1988, Asai 2002, Sato 2002, Kimura *et al*. 2003, Bai *et al*. 2006). However, no empirical studies have explored the exact mating patterns of these taxa. Understanding mating systems and patterns in these taxa will provide important insight into the ecological role of this mating system.

Here we characterize microsatellite genetic markers for parentage analysis and examine mating patterns of *A. mono* within a natural forest stand. We address the following questions: (1) is flowering of the two mating types, protandry and protogyny, in *A. mono* reciprocal and synchronous? (2) Does heterodichogamy in *A. mono* effectively avoid selfing (3) and promote between-type mating? (4) What other factors affect mating patterns?

Another issue in this study is the effects of forest fragmentation on the mating system and gene flow in *A. mono*. However, we could not perform direct paternity testing on seeds in fragmented populations to compare with the above data from continuous populations because of our experimental restrictions. Here we employ indirect methods to estimate the levels of gene flow in conserved and fragmented populations based on the genetic structure of adult trees, and predict the possible effects of forest fragmentation on gene flow and genetic diversity.

Materials and Methods

The species

Acer mono Maxim. var. *marmoratum* (Nichols.) Hara f. *dissectum* (Wesmael) Rehder (hereafter *A. mono*) is a deciduous tree species that reaches a height of about 20 m and is one of the major components of cool-temperate forests in Japan. Sex expression in *A. mono* was first described in detail by Mitigami *et al.* (1989); males and two types of hermaphroditic (protandrous and protogynous) individuals were found in a population. This maple is known to be visited by various types of insects such as flies, hover flies, and small solitary bees of the Halictidae and Andrenidae (Matsui 1991) and is considered to have generalist pollination systems.

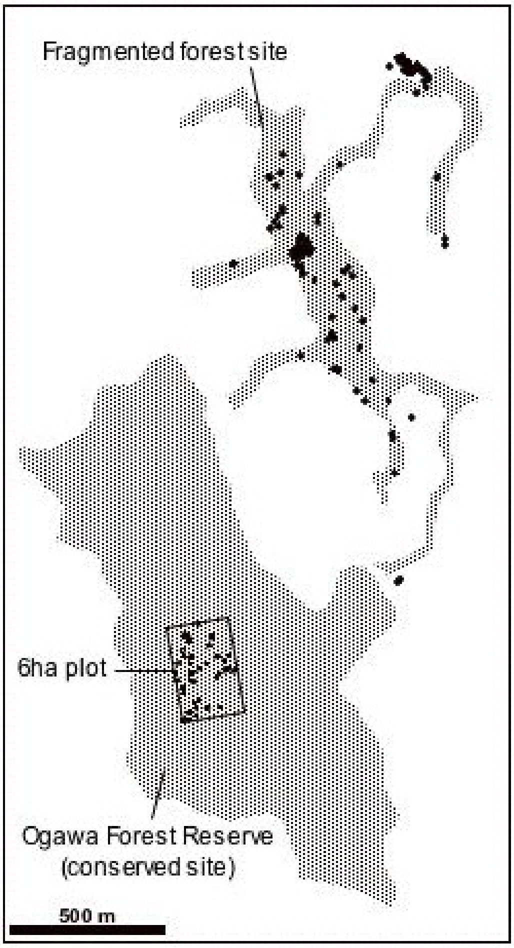


Fig. 1. Map of the study sites. The shaded area indicates natural forests in the conserved and the fragmented sites. Dots show the locations of sampled individuals of *A. mono*.

The field study and sampling

The field study was conducted in a 6-ha plot in Ogawa Forest Reserve (36˚56' N, 140˚35' E, 610-660 m above sea level) and in a neighboring fragmented forest site in Ibaraki and Fukushima Prefecture (Fig. 1). All trees with diameter at breast height (DBH) >5 cm in the 6-ha plot had been tagged and mapped, and DBH of each tree had been measured every four years.

All tagged individuals of *A. mono* in the 6-ha plot were checked for anthesis and flowering type (male, protandry, or protogyny) in 2003. In 2005, flowering phenology and sex expression of all flowering trees in the plot were examined; the sex of flowers in bloom was recorded for each flowering tree in the plot every 3-5 days from 5 to 20 May by collecting three to five inflorescences. Sex phases of flowering individuals at a given time were classified into three categories including female, male, and cosexual stages. The flowering rate of a single plant was estimated by the proportion of flowering shoots in its crown. Potential mating probability P(*i*, *j*), which refers to the degree of temporal overlap between the male stages of individuals with flowering type *i* and the female stages of those with flowering type *j*, was calculated following the methods of Sato (2002).

Leaf samples for genetic analysis were collected from all 46 flowering trees and eight non-flowering trees from the 6-ha plot, and from 130 flowering individuals from the fragmented forest site. Seeds for paternity analysis were collected in October 2003 from four maternal trees in the 6-ha plot, including one protandrous (tree number *PP6587*) and three protogynous (*PP4762*, *PP5249*, *PP6487*) individuals. To detect pollen flow from nearby areas outside the plot, additional leaf samples were collected from 33 flowering trees in the surrounding area with a width of 50 m outward from each side of the 6-ha plot.

Development of microsatellite markers in **Acer mono**

We characterized nuclear microsatellite markers in *A. mono* as described in Kikuchi & Shibata (in press). Microsatellites were developed from a genomic library enriched for (CT) dinucleotide repeats in *A. mono* Maxim. var. *marmoratum* (Nichols.) Hara and screened for PCR amplification and polymorphism using 34 individuals from the 6-ha plot. Thirteen polymorphic loci were characterized, with an average of 13.8 alleles per locus (Table 1).

Genetic analysis

Six (Am116, Am118, Am607, Am742, Am775, and Am909) of the developed microsatellite markers were employed in further analysis according to their robustness of PCR amplification, their reliability of allele scoring, and the low frequency or absence of null alleles. Moreover, we used two microsatellite loci, MAP09 (Pandey *et al.* 2004) and Aca24 (Terui *et al.* 2006), which have been reported in other *Acer* species (*A. pseudoplatanus* and *A. capillipes*, respectively).

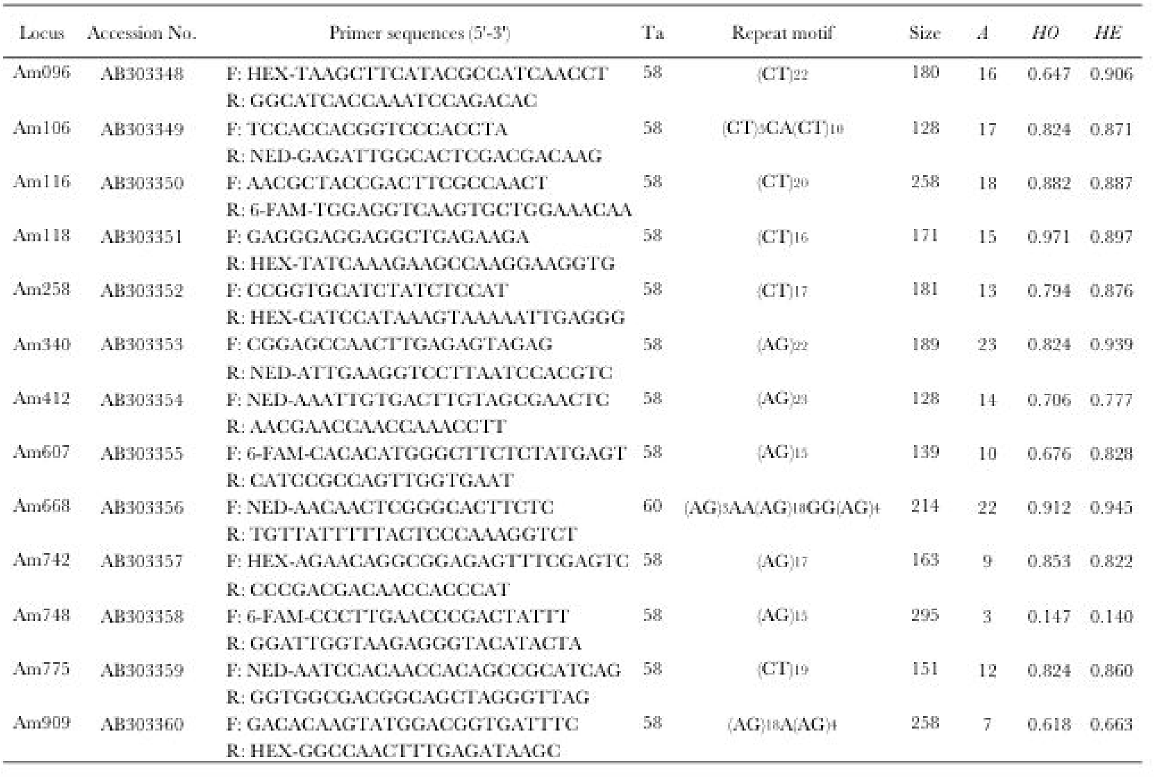
DNA samples of adult individuals were extracted from leaf tissues using the cetyltrimethylammonium bromide (CTAB) method described by Murray & Thompson (1980) and modified by Mukai & Yamamoto (1997). DNA from seeds was extracted using a DNeasy Plant Mini Kit (Qiagen). PCR amplifications were performed in a 6-μL reaction mixture consisting of approximately 1 ng of template DNA, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl2, 0.2 mM of each dNTP, 0.25 μM of each primer, and 0.25 U *Taq* polymerase. The PCR conditions included an initial denaturation step of 3 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at an annealing temperature of 50 to 60°C (for details see Table 1), and 30 s at 72°C, followed by a final extension step of 5 min at 72°C. Alleles were scored on an ABI 3100 automated sequencer, using the software GeneScan 3.7.1 and Genotyper 2.5 (Applied Biosystems).

Genetic data analysis

Estimators of microsatellite genetic diversity, measured as the number of alleles per locus (N_A) and expected heterozygosities (H_E), were computed for two groups of adult trees, one from the 6-ha plot and the other from the fragmented forest, using the program GenAlex (Peakall & Smouse 2006). The inbreeding coefficient F_{IS} and the fixation index F_{ST} were calculated according to Weir & Cockerham (1984) using FSTAT software (Gouldet 2001).

Genotype data of adult individuals from the 6-ha plot and the fragmented forest were also used to examine spatial genetic structure. We employed a spatial autocorrelation analysis between individuals based on multilocus genotypes. A relationship coefficient *r* (Wang 2002) was computed using SPAGeDi 1.2 software (Hardy & Vekemans 2002). Ten distance classes up to 303.7 m and 15 up to 1277.4 m were automatically set up at irregular distance intervals using SPAGeDi to allocate a relatively equal number of individual pairs to each distance class.

Table 1. Characteristics of 13 microsatellite loci in *Acer mono*. These include the locusname, DDBJ accession number, primer sequence, and annealing temperature (T_a). Repeat motif and expected product size are derived from the sequenced clone. Number of alleles (A) and observed (H_O) and expected (H_E) heterozygosity were based on 34 samples (cited from Kikuchi & Shibata in press).



Locus	Accession No.	Primer sequences (5'-3')	Ta	Repeat motif	Size	*A*	*HO*	*HE*
Am096	AB303348	F: HEX-TAAGCTTCATACGCCATCAACCT R: GGCATCACCAAATCCAGACAC	58	$(CT)_{22}$	180	16	0.647	0.906
Am106	AB303349	F: TCCACCACGGTCCCACCTA R: NED-GAGATTGGCACTCGACGACAAG	58	$(CT)_5CA(CT)_{10}$	128	17	0.824	0.871
Am116	AB303350	F: AACGCTACCGACTTCGCCAACT R: 6-FAM-TGGAGGTCAAGTGCTGGAAACAA	58	$(CT)_{20}$	258	18	0.882	0.887
Am118	AB303351	F: GAGGGAGGAGGCTGAGAAGA R: HEX-TATCAAAGAAGCCAAGGAAGGTG	58	$(CT)_{16}$	171	15	0.971	0.897
Am258	AB303352	F: CCGGTGCATCTATCTCCAT R: HEX-CATCCATAAAGTAAAAATTGAGGG	58	$(CT)_{17}$	181	13	0.794	0.876
Am340	AB303353	F: CGGAGCCAACTTGAGAGTAGAG R: NED-ATTGAAGGTCCTTAATCCACGTC	58	$(AG)_{22}$	189	23	0.824	0.939
Am412	AB303354	F: NED-AAATTGTGACTTGTAGCGAACTC R: AACGAACCAACCAAACCTT	58	$(AG)_{23}$	128	14	0.706	0.777
Am607	AB303355	F: 6-FAM-CACACATGGGCTTCTCTATGAGT R: CATCCGCCAGTTGGTGAAT	58	$(AG)_{15}$	139	10	0.676	0.828
Am668	AB303356	F: NED-AACAACTCGGGCACTTCTC R: TGTTATTTTTACTCCCAAAGGTCT	60	$(AG)_3AA(AG)_{18}GG(AG)_4$	214	22	0.912	0.945
Am742	AB303357	F: HEX-AGAACAGGCGGAGAGTTTCGAGTC R: CCCGACGACAACCACCCAT	58	$(AG)_{17}$	163	9	0.853	0.822
Am748	AB303358	F: 6-FAM-CCCTTGAACCCGACTATTT R: GGATTGGTAAGAGGGTACATACTA	58	$(AG)_{15}$	295	3	0.147	0.140
Am775	AB303359	F: NED-AATCCACAACCACAGCCGCATCAG R: GGTGGCGACGGCAGCTAGGGTTAG	58	$(CT)_{19}$	151	12	0.824	0.860
Am909	AB303360	F: GACACAAGTATGGACGGTGATTTC R: HEX-GGCCAACTTTGAGATAAGC	58	$(AG)_{18}A(AG)_4$	258	7	0.618	0.663

Paternity of seeds collected from four trees in the 6-ha plot was determined using the program Cervus version 3.0 (Marshall *et al*. 1998, Kalinowski *et al*. 2007). We used likelihood methods implemented in Cervus to find the most likely pollen parents. Cervus calculates likelihood ratios (the likelihood that the candidate parent is the true parent divided by the likelihood that the candidate parent is not the true parent) and LOD scores (the natural log of the product of the likelihood ratios at each locus). Paternity was assigned to an individual with the highest LOD scores and Delta values (difference in LOD scores between the most likely parent and the second most likely parent) exceeding the 90% confidence level. If none of the candidates in the 6-ha plot plus its surrounding area (a total of 12 ha) had positive LOD scores, seeds were scored as being sired by an individual outside the population. In any other case, paternity was identified as "not determined."

Results

Flowering phenology in **A. mono**

Forty-six out of 173 tagged trees of *A. mono* (DBH >5 cm in 2001) in the 6-ha area were found bearing flowers during the study period. Most (95.2%) of the trees with DBH >20 cm produced flowers, whereas none of the trees with DBH <15 cm flowered (Fig. 2; data based on Forestry and Forest Products Research Institute 2003). Sex expression of all flowering individuals in the plot was investigated in 2005, and 25 trees were found to be protandrous and 21 were protogynous. Male individuals were not found in the plot in 2005. Female and male flowering in the protogynous and the protandrous individuals, respectively, were at their peak in early May; female flowers of 17 (81%) protogynous individuals and male flowers of 20 (80%) protandrous individuals were in bloom from 5 to 12-13 May. The peak in male flowering of the protogynous individuals occurred from 16 to 17 May and was synchronized with the female-flowering peak of protandrous individuals (Fig. 3); during this period, 19 protogynous individuals had male flowers in bloom, of which two were in the cosexual stage, and 20 protandrous individuals were in the female stage (Fig. 3). The second male stage was observed in 12 protandrous individuals in mid to late May, after the female phase.

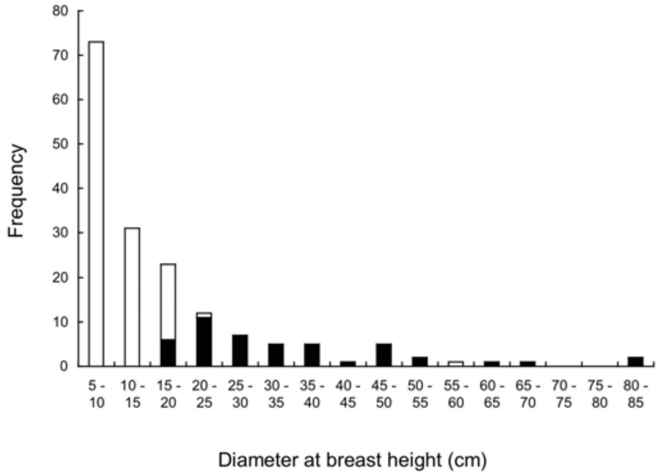


Fig. 2. Size-class distribution of *A. mono* trees with DBH >5 cm in the 6-ha plot, based on data collected in 2001 (Forestry and Forest Products Research Institute 2003). Filled and open bars indicate trees found bearing and not bearing flowers, respectively.

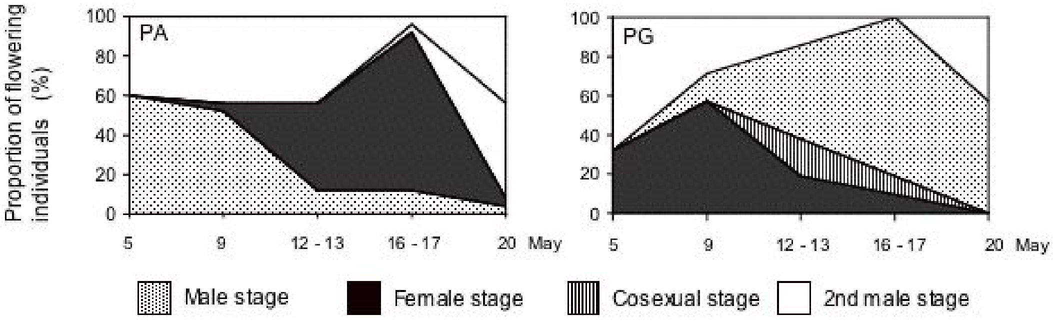


Fig. 3. The proportion of flowering individuals in each flowering phase at a given time of observation for protandrous (PA, above) and protogynous (PG, below) individuals.

The potential mating probability was 0.71 for between-type mating, 0.40 for P (PG, PA) and 0.31 for P (PA, PG), where PA and PG denote protandrous and protogynous individuals, respectively. The potential probability for within-type mating was 0.29, 0.10 for P (PA, PA) and 0.19 for P (PG, PG).

Genetic diversity and structure

Using eight microsatellites, 128 alleles were detected in 211 samples. An average of 13.25 alleles per locus were found in 81 samples from the 6-ha plot plus its surrounding area in the conserved site, whereas N_A was 14.25 in 130 samples from the fragmented site (Fig. 1). H_E was 0.801 and 0.781 in samples from the conserved and the fragmented sites, respectively. F_{IS} values were not significantly different from the null hypothesis in the former (F_{IS} = -0.007), but were significantly positive (F_{IS} = 0.031, p <0.05) in the latter. F_{ST} was 0.029 (95% confidence interval of 0.013-0.047), suggesting low but significant genetic divergence between the two sites.

The relationship coefficient *r* was significantly positive for the four shortest distance classes (<101.7 m) and negative at six of the eight longest distance classes (>334.4 m) for the fragmented forest site at the 95% significance level. It was positive and negative only in the shortest (<44.7 m) and the longest distance classes (213.8-303.7 m) in the 6-ha plot (Fig. 4).

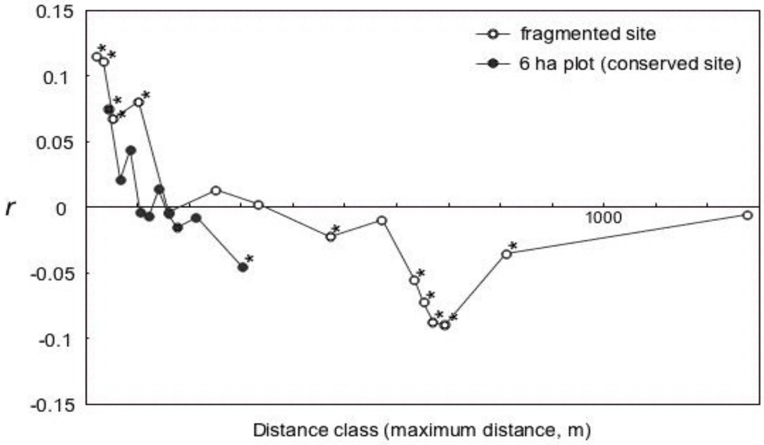


Fig. 4. The results of spatial autocorrelation analysis within the 6-ha plot in the conserved site (filled circles) and in the fragmented site (open circles). Asterisks indicate significant values for relationship coefficients *r* (p <0.05) based on 1000 permutations.

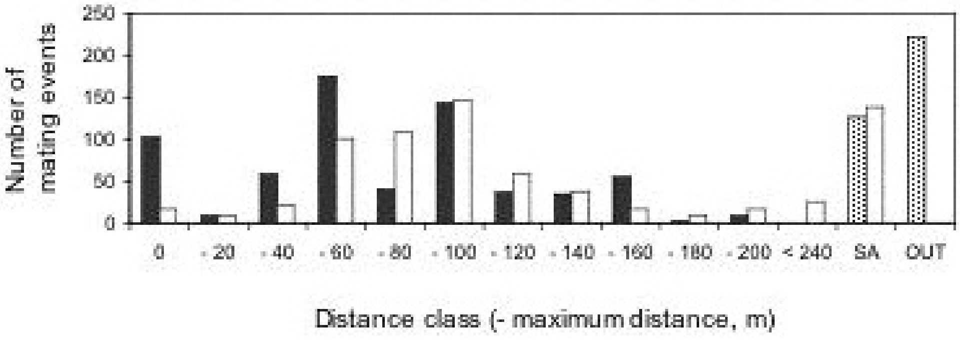


Fig. 5. Distribution patterns of mating distance inferred from parentage analysis. Filled and shaded bars indicate the number of mating events in each distance class. SA and OUT denote the frequency of seeds sired by individuals from the surrounding area and the frequency of seeds in which paternity was not decided, respectively. Open bars indicate expected values based on the number of individual pairs at a given distance class.

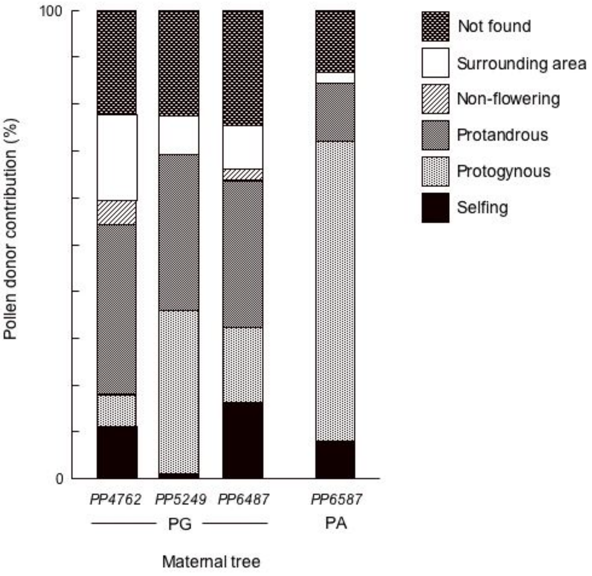


Fig. 6. The proportion of selfed seeds and outcrossed seeds sired by each type of pollen donor (protogynous and protandrous individuals, those not found bearing flowers in the plot [shown as "Non-flowering"], and those in the surrounding area). The proportion of seeds without a pollen parent among the sampled adults is indicated as "Not found."

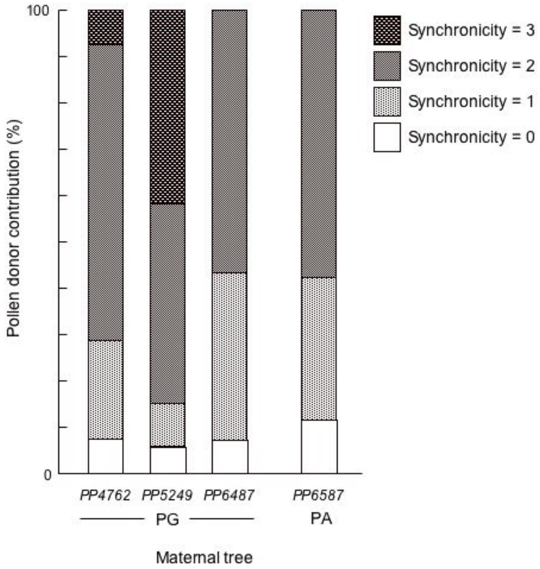


Fig. 7. The relative contribution of each type of pollen donor among the outcrossed seeds sired by flowering adults within the plot, in relation to flowering synchronicity between the male phase of pollen donors and the female phase of maternal trees.

Parentage analysis

A total of 1036 seeds collected from four mother trees were genotyped for paternity tests. Paternity was assigned to individuals within the plot in 60.6% of the seeds and to individuals in the surrounding area in 12.1% of seeds, and 21.4% were assumed to be sired by outside individuals. Paternity was not determined for only 0.29% of the seeds. The distribution of mating distance was illustrated in Fig. 5. Selfing rates ranged from 0.01% for the maternal tree *PP5249* to 15.6% for *PP6487* (Fig. 6). Of 554 outcrossed progenies sired by flowering trees in the plot, 396 were sired by trees of the reciprocal morph, significantly exceeding the number sired by the same heterodichogamous morph ($p < 0.0001$, Chi-square test). However, between-type mating was largely superior to within-type mating for three of the maternal trees, *PP4762*, *PP6587* ($p < 0.001$), and *PP6487* ($p < 0.01$), but not for *PP5249*.

Next, we examined the effects of reciprocal flowering synchronicity between pollen donors and mother plants on mating patterns. The degree of reciprocal synchronicity between a maternal tree and each pollen donor was numerically scored from 0 to 3 based on the number of observations at which the female flowering of the maternal tree coincided with the male flowering of the pollen donor. Contribution of pollen donors with flowering synchronicity ≥1 (i.e., with male flowering period overlapping female flowering of the maternal tree for at least one observation) was disproportionately higher for all the maternal trees ($p < 0.001$, chi-square test; Fig. 7).

To address the factors determining mating patterns in *A. mono*, the relative contribution of pollen donors was regressed with their flowering synchronicity to a maternal tree, mating distance, and their flower production. The relative flower production of each individual was roughly estimated from its crown size as determined from DBH and the proportion of flowering shoots in the crown. Here we applied a simple nonlinear power function model relating crown width (*CW*) and DBH of the congener *A. rubrum* (Bragg 2001):

$$CW = 1.64 + 0.249\ (\mathrm{DBH})^{0.876}.$$

The estimation of relative flower production was therefore obtained by multiplying $(CW)^{*}2\pi/4$ and the proportion of flowering shoots. Pollen donor contribution was correlated with relative flower production ($r = 0.550$, $p < 0.001$) and flowering synchronicity ($r = 0.349$, $p < 0.001$), but not with mating distance (Fig. 8).

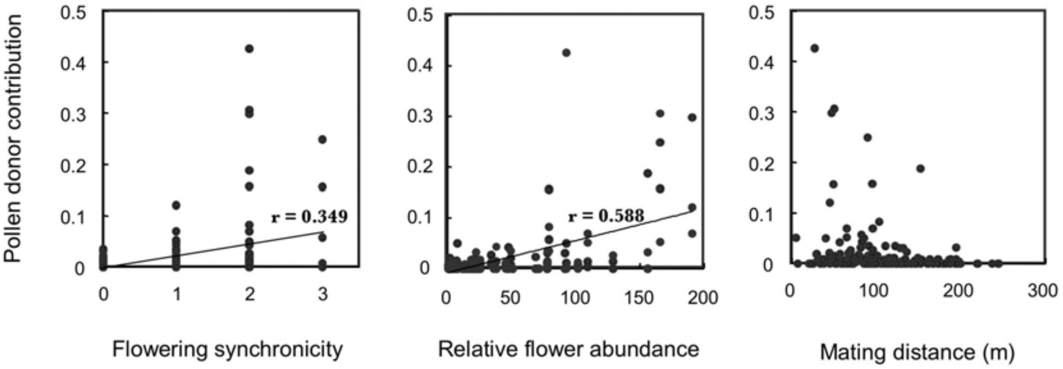


Fig. 8. Relationships between the contribution of pollen donors and their flowering synchronicity with the maternal tree, their flower abundance, and mating distance.

Discussion

Previous studies on flowering systems of heterodichogamous taxa have revealed reciprocal and synchronous flowering of protandrous and protogynous mating types within a population (Gleeson 1982, Pendleton *et al*. 1988, Asai 2002, Sato 2002, Kimura *et al*. 2003, Bai *et al*. 2006), and heterodichogamy has been considered an effective breeding system to avoid selfing and to ensure outcrossing by promoting between-type mating.

This study investigated exact mating patterns in *A. mono* and serves as an important empirical case addressing the ecological role of heterodichogamy in this maple.

In this study, flowering phases of the two mating types in *A. mono* were synchronous and reciprocal (Fig. 3). The potential mating probability, which was 2.4 times higher between the mating types than within the same type, is comparable with that of *A. japonicum* (Sato 2002). Temporal separation of male and female flowering within individual trees was apparent, but not perfect, with a cosexual stage in a few protogynous trees, as has been reported in previous studies (Pendleton *et al*. 1988, Sato 2002 and Kimura *et al*. 2003).

Paternity analysis using highly polymorphic microsatellites demonstrated high levels of outcrossing in *A. mono*. However, a certain amount of self-fertilization occurred in this natural population, suggesting no obligate self-incompatibility systems in *A. mono*. Self-compatibility is considered common in heterodichogamous taxa, including *Juglans* and *Acer* (reviewed by Renner 2001). Gabriel (1966) reported self-compatibility without a gametophytic or sporophytic incompatibility system in the heterodichogamous maple *A. saccharum* by experimental pollination, but also demonstrated lower seed set, probably resulting from post-zygotic abortion. Thus, the selfing rate found in this study may be an underestimate of self-fertilization in the natural population.

Self-fertilization in a natural population requires overlap of the male (or pollen remaining viable) and the female stages (or stigmas remaining receptive) within individuals. Therefore, it is considered that the degree of temporal overlap between male and female functions within individuals may be an important factor contributing to the variable selfing rates among maternal trees (0.01-16.2%). Specifically, the maternal tree *PP4762* with 9.6% of seeds self-fertilized showed a cosexual stage, in which female flowers remained in bloom in the upper layer of the tree while male flowers occurred in the lower layer. Although our observations in this study detected cosexual stages only in a few protogynous individuals, Sato (2002) described cosexual stages in protandrous individuals of *A. japonicum* between the male phase and the second male phase, suggesting a probability of self-fertilization in protandrous individuals. We conclude that the apparent but imperfect segregation of male and female flowering periods ensures high outcrossing in *A. mono*.

The hypothesis of between-type mating was not supported for all of the maternal trees (Fig. 6). Otherwise, a higher contribution of pollen donors just requires synchronicity between male flowering of pollen donors and female flowering of maternal trees (Fig. 7). Specifically, in the maternal tree PP5249, the female flowering phase lasted until the male flowering periods of many of the protogynous individuals in the plot, resulting in high pollen contribution from the same flowering type. Subsequently, the ratio of between-type mating to within-type mating was 2.94, which exceeded the expected value of potential mating probability (2.44).

Other than flowering synchronicity, flower production of pollen donors was considered a factor that increases their pollen contribution (Fig. 8). Interaction of these factors may well explain mating patterns of *A. mono* within this plot. We did not detect a significant negative relationship between distance and effective pollen dispersal. Effective pollination occurred over distances greater than 150 m (Fig. 8). Moreover, although the number of mating events dropped at distances greater than 180 m, more than 30% of the seeds were still sired by individuals not found within the plot (Fig. 5). Extending the spatial scale of paternity testing will be required to capture the negative effect of distance on pollen dispersal.

Analysis of spatial genetic structure provides an indirect estimate of the levels of gene flow via pollen and seeds. Spatial autocorrelation analysis in this study clarified significant genetic structure both within the 6-ha plot in the conserved site and within the fragmented site. The x-axis intercepts, where the relationship coefficient r first crossed the x-axis, suggest that gene flow of *A. mono* becomes restricted at the geographical scale of about 100-300 m. In the fragmented site, high r-values at distance classes up to 100 m represent spatial clustering of genetically related individuals in two younger forest stands with high density of this maple (Fig. 1). Moreover, the inbreeding coefficient F_{IS} was significantly positive in the fragmented site, whereas it did not depart from the null hypothesis in the conserved site. This evidence suggests reduced gene flow of *A. mono* throughout the fragmented forest.

Effective pollination should occur over hundreds of meters; however, isolation of reproductive individuals of *A. mono* by forest fragmentation may reduce the availability of suitable pollen donors with reciprocal flowering synchronicity and flower abundance. This study suggests two possible scenarios: increased selfing, if female and male functions temporarily overlap within individuals, and reduced seed production, if such overlap does not occur. Further analysis of the exact mating patterns within fragmented sites should be conducted.

References

Asai T (2000) Dichogamy in fullmoon maple (*Acer japonicum* THUNB.). *Bulletin of the Hokkaido Forestry Research Institute* **37**: 27-40 (in Japanese).

Bai W, Zeng Y, Liao W, Zhang D (2006) Flowering phenology and wind-pollination efficacy of heterodichogamous *Juglans mandshurica* (Juglandaceae). *Annals of Botany* **98**: 397-402.

Bragg DC (2001) A local basal area adjustment for crown width prediction. *Northern Journal of Applied Forestry* **18**: 22-28.

de Jong PC (1994) Taxonomy and reproductive biology of maples. In: *Maples of the World* (eds. Van Gelderen DM, de Jong PC, Oterdoom HJ), pp. 69-103. Timber Press, Portland, OR, USA.

Forestry and Forest Products Research Institute (2003) Forest Dynamics Database. http://fddb.ffpri-108.affrc.go.jp/ Viewed 15 August 2007.

Gabriel WJ (1966) Reproductive behavior in sugar maple: self-incompatibility, cross-compatibility, agamospermy, and agamocarpy. *Silvae Genetica* **16**: 165-168.

Gleeson SK (1982) Heterodichogamy in walnuts: inheritance and stable ratios. *Evolution* **36**: 892-902.

Gleiser G, Verdú M (2005) Repeated evolution of dioecy from androdioecy in *Acer*. *New Phytologist* **165**: 633-640.

Gouldet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). http://www.unil.ch/izea/softwares/fstat.html.

Hardy OJ, Vekemans X (2002). SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes* **2**: 618-620.

Kalinowski ST, Taper ML & Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology* **16**: 1099-1006.

Kikuchi S, Shibata M (2007) Development of polymorphic microsatellite markers in *Acer mono* Maxim. *Molecular Ecology Notes* (in press).

Kimura M, Seiwa K, Suyama Y, Ueno N (2003) Flowering system of heterodichogamous *Juglans ailanthifolia*. *Plant Species Biology* **18**: 75-84.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* **7**: 639-655.

Mitigami Y, Mizui N, Kikuzawa K (1989) Flowering patterns and fruit characteristics of *Acer mono. Transactions of the Meeting in Hokkaido Branch of the Japanese Forestry Society 37: 30-32 (in Japanese).*

Mukai Y, Yamamoto N (1997) DNA and RNA isolation method of woody plants. In: *PCR Experimental Protocols* *for Plants* (eds. Shimamoto K, Sasaki T), pp. 57-62. Shujunsha, Tokyo (in Japanese).

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research* **8**: 4321-4325.

Pandey M, Gailing O, Fischer D, Hattemer HH, Finkeldey R (2004) Characterization of microsatellite markers in sycamore (*Acer pseudoplatanus* L.). *Molecular Ecology Notes* **4**: 253-255.

Peakall, R, Smouse PE (2006) GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288-295.

Renner SS (2001) How common is heterodichogamy? *Trends in Ecology and Evolution* **16**: 595-597.

Sato T (2002) Phenology of sex expression and gender variation in a heterodichogamous maple, *Acer japonicum*. Ecology **83**: 1226-1238.

Terui H, Lian CL, Saito Y, Ide Y (2006) Development of microsatellite markers in *Acer capillipes*. *Molecular Ecology Notes* **6**: 77-79.

Wang J. 2002. An estimator for pairwise relatedness using molecular markers. *Genetics* **160**: 1203-1215.

Weir BS, Cockerham CC 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution* **38**: 1358-1370.

The English in this document has been checked by at least two professional editors, both native speakers of English. For a certificate, see: http://www.textcheck.com/cgi-bin/certificate.cgi?id=N67grw

Effects of Human Disturbance of Forest on Reproduction of a Heterodichogamous Maple, *Acer mono*

Mitsue Shibata[1], Satoshi Kikuchi[2], Hiroshi Tanaka[2], Hiroshi Yoshimaru[2], Kaoru Niiyama[2]

[1]Tohoku Research Center, Forestry and Forest Research Institute,
Morioka, Iwate, 020-01, Japan

[2]Forestry and Forest Products Research Institute, Matsunosato 1,
Tsukuba, Ibaraki 305-8687, Japan

Introduction

There is concern about the effect of human disturbance of forests on trees' reproductive success, which underpins forest ecosystems. To predict that effect, it is necessary to investigate and analyze factors affecting plant reproduction. One of the factors, local population density, which is generally altered by anthropogenic forest use, is particularly important. Many studies report low population density causing low pollination success due to there being less visitation of pollinators to flowering plants (Kunin 1997, Ghazoul and McLeish 2001, Wagenius 2006), fewer mating individuals, and inbreeding depression due to the receipt of low-quality pollen (Bosch and Waser 1999). These negative effects on pollination efficiency reduce seed set (House 1992, Aizen and Feinsinger 1994, , Cascante et al. 2002). However, several studies have showed positive effects of low population density on other reproductive stages, such as low seed predation or fungal attack (Cascante et al. 2002, Fujimori et al. 2006).

In addition to population density, other factors such as tree size may have large effect on reproduction. Size-dependent resource allocation, whereby larger plants can invest more resources in fruits and seeds, has been observed in several plant species (Ohlson 1988, de Jong and Klinkhamer 1989). Such seeds may have higher levels of soundness. Since larger plants generally have larger floral displays, they will attract more pollinator insects (Klinkhamer et al. 1989), and may have better pollination efficiency.

Many plant species of a wide variety of taxa have dichogamous flowering systems. Sex expression is also an important reproductive factor for these species. For instance, in heterodichogamous species, which have a mixture of protogynous (stigmas are receptive before pollen is shed) and protandrous (pollen is shed before stigmas are receptive) mating types in a population (Renner 2001), resource investment to fruit maturation was higher in protogynous types than protandrous types (Asai 2000, Sato 2002). It is thought that heterodichogamous plants have reciprocal mating among different sex morph types (Gleeson 1982, Dommee et al. 1990, Kimura et al. 2003) to avoid self-pollination (Cruden and Hermann-Parker 1977) and reduce self-interference (Lloyd and Webb 1986). If such pollination systems are common, individuals are limited to mating with others of reciprocal sex morph, and their pollination efficiency will be more affected by spatial distribution of the reciprocal sex morph type rather than by the overall population density.

Thus, when we consider effect of human disturbance of forest on reproduction of trees, we should assess the effect of gradient of local population on reproductive success of trees together with the effect of other factors mentioned above. However, reproductive consequences of negative or positive impact of those factors are not yet well known, especially for trees of temperate forests. Therefore, we investigate the whole fruiting processes of *A. mono* Maxim. var. marmoratum (Nichols.) Hara f. dissectum (Wesmael) Rehder, which is a temperate forest component. This species is known to have an insect pollination system and heterodichogamous flowers as other *Acer* species. Our objective of this study is to clarify which stages of the fruiting process are affected by local population density, sex morph or individual tree size.

Species

Acer mono Maxim. var. marmoratum (Nichols.) Hara f. dissectum (Wesmael) Rehder (hereafter *A. mono*) is one of main canopy component trees in cool temperate deciduous forests in Japan. Inflorescences commonly form a compound umbel with two types of small flowers. One is a male flower with dehisced anthers and no pistil or an atrophied (sterile) pistil. The other is a female flower with indehiscent anthers and a healthy (fertile) pistil. In natural populations, a few trees have only inflorescences with male flowers and most trees have one of three types of inflorescence: (1) female flowers bloom before male flowers (female-male), (2) male flowers bloom before female flowers (male-female), or (3) male flowers bloom before and after female flowers (male-female-male). In this paper, we term (1) the protogynous (PG) type, and (2) and (3) the protandrous (PA) type. In most trees, the blooming period of each type of flower is synchronized at the individual tree level (Shibata M. personal observation). Thus, each tree has generally a male phase and a female phase in a flowering season. Various types of insects, such as flies, hoverflies and small solitary bees (Halictidae, Andrenidae) visit the flowers of this species (Matsui 1991). Thus, this species is a generalist plant; i.e. pollinated by several or many species from different taxa.

Study site

The study was carried out at Ogawa Forest Reserve (OFR) and neighboring fragmented forest, located in the southern part of the Abukuma Mountains, central Japan. The core area of OFR (ca. 100 ha) is an old-growth temperate deciduous forest. It is known that the land surrounding OFR has suffered human disturbance such as forest burning in the past (Suzuki 2002). Although OFR has been protected for at least 80 years, most of the edge area of this forest reserve is secondary forest likely to have been affected by human disturbance. Species composition and structure in the fragmented forest areas are similar to OFR (Masaki T. unpublished). The fragmented forest areas also have both old-growth and secondary forest stands. Information about the spatial location of OFR and the neighboring fragmented forest areas is shown in Isagi et al. (2007) and Fujimori et al. (2006)

Methods

Mapping of reproductive adult trees

We marked all flowering trees of *A. mono* as adult trees in a 6 ha plot of OFR and the fragmented forest areas in 2002. Canopy condition (canopy, suppressed, in gap, or gap edge), sex morph, and DBH of each tree were recorded. In order to determine the sex morph (PA or PG) of each tree, several inflorescences were collected in mid flowering season, and the condition of male and female flowers (bud, flowering, wilted, or dead) in the inflorescences was observed to determine the sexual sequence of flowering.

Since forest management differed between OFR and the fragmented forest, there was a certain degree of gradient of local population density. The geographical position of each tree was recorded on a map of the area. Based on this map, sample trees from sites of different local population densities were selected. For each sample tree, the average distance from the nearest five adults was measured as an index of local population density. The average distance from the nearest five adults was measured in two ways: DIS-N, distance from the nearest adults irrespective of sex morph, and DIS-RS, distance from the nearest adults that have the reciprocal sex morph (for instance, the distance from the nearest five PG trees was measured for PA trees).

Comparing seed demography

In order to compare reproductive performance between canopy trees with different local population density, individual tree size, and sex morph, we sampled seeds from 17 trees in 2002, 23 trees in 2003, and 40 trees in 2005. Most seeds were collected directly from branches just before the seed fall season (late October). The number of sampled seeds was from 100 to 500 for each tree. All seeds were cut and their kernel condition was examined, categorizing them according to condition as empty, subjected to predation by insects, decayed, or sound. Percentages of these seed conditions were calculated for each tree. Other than these parameters, seed size and percentage of immature seed fall were measured in 2003 and 2005, respectively. Average kernel weight of a sound seed as an index of seed size was measured for 12 of 23 sample trees in 2003. Percentage of immature seed fall was estimated for 30 of 40 trees in 2005.

Factors affecting seed production were analyzed by multiple regression analysis. The explaining variables were 3 factors: DIS-N or DIS-RS as an index of local population density, DBH as individual tree size, and type of sex morph. DIS-N and DIS-RS were transformed to log scales. The dependent variables were 6 demographic parameters: percentage of immature seed fall, empty seeds, seeds subjected to predation by insects, decayed seeds, sound seeds, and seed kernel weight. For the percentage of empty seeds, seeds subjected to predation by insects, decayed seeds, and sound seeds, data were pooled among the 3 observation years to enlarge sample size and reveal overall trends including annual variation of seed crop.

Results

We constructed two multiple regression models, one had DIS-N, DBH, and sex morph for the explaining variables, and the other had DIS-RS, DBH, and sex morph. The latter had a lower value of AIC (Akaike's Information Criterion) than the former for all the 6 dependent variables, suggesting that DIS-RS was a clearer index than DIS-N for the effect of local population density.

Distance from conspecific adults had a significant positive correlation with the percentage of immature seed fall and empty seeds (Tab. 1), but a negative correlation with the percentage of seeds subjected to predation and decayed seeds. Distance from conspecific adults did not have significant correlation with the percentage of seed soundness. However, its positive effect on seed kernel weight was marginally significant. Sex morph was only related to immature seed fall. Trees of PG type had a significantly lower percentage of immature seed fall than trees of PA type (Tab. 1). No effect was detected for size of individual trees (Tab. 1). These trends in the explaining variables were the same for the two models.

Discussion

Factors affecting pollination efficiency

The positive effect of the distance of conspecific adults on the percentage of immature seed fall and empty seeds (Tab. 1) suggested pollination efficiency increasing with higher local population density. We expected that a larger plant would have higher pollination efficiency because of the larger number of flowers to attract more pollinator insects (Klinkhamer et al. 1989). However, the percentages of immature seed fall and empty seeds were not affected by tree sizes but were instead influenced by local population density (Tab. 1). This indicates that the number of flowers at the local population level (several hundred square meter scale) was more important for pollination success than that at the individual level (several dozen square meter scale) for *A. mono*.

In contrast to *A. mono*, pollination efficiency of *Kalopanax pictus,* which is also a main component tree species in this forest, was not affected by local population density (Fujimori et al. 2006). We presume that such different responses between tree species reflect differences in pollinator foraging range and abundance. The most frequent flower visitors of *K. pictus* is the honeybee, which has a large foraging range (Fujimori et al. 2006); its foraging distance can range 1-10 km (Visscher and Seeley 1982; Sasaki 1999). Honeybees contributed to effective pollination in sparsely-distributed *Dinizia excelsa* trees (distance between trees was ca. 1500 m) in fragmented forests in Brazil (Dick et al. 2003). Honeybees in this forest may also contribute to effective pollination for *K. pictus*. The most frequent flower visitors of *A. mono* were small solitary bees, hoverflies, and flies. Such insects probably work as pollinators. They showed large variations in abundance and species composition with landscape change even over small distance (Steffan-Dewenter et al. 2002, Sueyoshi et al. 2003), indicating that small solitary bees and flies have a narrower foraging area and are sensitive to changes in stand conditions. We presume that pollination efficiency of *A. mono* varies with local population density due to changes in pollinator abundance and behavior in responding to the local flowering tree density.

We expected that local population density of the reciprocal sex morph rather than that of both types may have a stronger effect on pollination for *A.mono*. However, the difference between them was not so large; AIC of the multiple regression models constructed with DIS-RS had a slightly lower value than that with DIS-N. This is probably caused by mixed spatial distribution of both sex morphs. Local population density was similar in both the models, and DIS-RS had a high correlation with DIS-N (r=0.94). It will depend on the degree of deflection of spatial distribution of each sex morphs whether local density of the reciprocal sex morph is more important or not.

Heterodichogamous trait was important as an individual level factor. Trees of PG type had significant lower immature seed fall than PA (Tab. 1). This result suggests that the former had higher efficiency of pollen acceptance (bias to female function) than the latter. Therefore, heterodichogamy relates not only to the reciprocal pollination system but also to the difference of gender specialization linked with sex morph. Several reports said that heterodichogamy was one of evolutional pathway from monoecy to dioecy (Pendleton et al. 2000, Sato 2002). The bias of female function in PG in this study may reflect such evolutional background. In contrast to PG, PA, which has higher immature seed fall, may expend effort to pollen supply like *Juglans ailanthifolia* (Kimura et al. 2003).

Density dependent seed mortality

Density dependent mortality has been well reported at the post-dispersal seed stage and seedling stage for many species (Shibata and Nakashizuka 1995, Hille Ris Lambers and Clark 2003, Wright et al. 2005). Our results showed density dependent mortality is also occurring at the pre-dispersal seed stage (Tab. 1) similar to other tree species that have been studied recently: *Samanea saman* in a tropical forest (Cascante et al. 2002), and *K. pictus* (Fujimori et al. 2006) and *Magnolia obovata* in a temperate forest (Isagi et al. 2007). We think that density dependent mortality is also a common phenomenon in the fruiting process.

Steffan-Dewenter et al. (2001) pointed out that decreasing predation at a low population density site counterbalances the disadvantage of the low pollination success. Our study also detected a similar situation. As a consequence of seed set, population density did not affect soundness of seeds in the spatial scale of this study (Tab. 1). However, we have to be careful not to give a simple explanation that local population density does not influence reproduction, because different functional groups, namely pollinators and seed predators, counterbalance their effects with respect to local population density. Unfortunately, we do not currently know the critical spatial scale of the habitats for these functional groups in detail. This study shows that the effect of local population density on plant reproduction was very complicated. We have to examine further the counterbalance between pollination and seed mortality and this will be part of an investigation including a wider range of the population densities of host plants.

Factors affecting seed maturation

Contrary to our expectations, individual tree size did not have a clear relationship with either seed soundness or seed size (Tab. 1). On the other hand, growth of the seed was affected by local population density. These results suggest that seed development of this species is regulated more by pollination than by individual resource conditions. However, seed size tends to be rather smaller at high local population density sites (Tab. 1). If neighboring individuals at the high local population density site are closely related to each other, and pollinators remain within the site, inbreeding depression among neighboring trees will occur. We presume that downsizing of seed results from such inbreeding depression. This means that high local population density has a negative effect on the quality of pollination like biparental inbreeding (Ritland and Jain 1981). To examine this suggestion, it is important to investigate mechanisms of the reproductive consequence related to local population by revealing the actual pollen flow by genetic approaches as a part of future study.

Conclusion

This study showed that local population density and sex morph had effects on various stages of the fruiting process. Impacts operated both negatively and positively. This complicated interaction may lead to autonomous control of abundance of this species, as with *Magnolia obovata* (Isagi et al. 2007). However, the response of each species depends on the variety of pollinators and seed predators as mentioned above. The response of local population density may also change with annual fluctuation of seed production and insect population dynamics. To consider effects of human disturbance of forest on tree reproduction, it is important to clarify plant-animal interaction in reproduction and in the long-term dynamics of target species.

References

Aizen MA, Feinsinger P (1994) Forest fragmentation, pollination, and plant reproduction in a chaco dry forest, Argentina. Ecology 75(2):330-351.

Asai T (2000) Dichogamy in fullmoon maple. (In Japanese with English summary) Bulletin of the Hokkaido Forestry Research Institute 37:27-40.

Bosch M, Waser NM (1999) Effects of local density on pollination and reproduction in *Delphinium nuttallianum* and *Aconitum columbianum*. American Journal of Botany 86(6):871-879.

Cascante A, Quesada M, Lobo JJ, Fuchs EA (2002) Effects of dry tropical forest fragmentation on the reproductive success and genetic structure of the tree *Samanea saman*. Conservation Biology 16:137-147.

Cruden RW, Hermann-Parker SM (1977) Temporal dioecism: an alternative to dioesism? Evolution 31(4):863-866.

Dick CW, Etchelecu G, Austerlitz F (2003) Pollen dispersal of tropical trees (*Dinizia excelsa*: Fabaceae) by native insects and african honeybees in pristine and fragmented Amazonian rainforest. Molecular Ecology 12:753-764.

Dommee B, Bompar JL, Denelle N (1990) Sexual tetramorphism in *Thymelaea hirsuta* (thymelaeaceae): evidence of the pathway from heterodichogamy to dioecy at the infraspecific level. American Journal of Botany 77(11):1449-1462.

Fujimori N, Samejima H, Kenta T, Ichie T, Shibata M, Iida S, Nakashizuka T (2006) Reproductive success and distance to conspecific adults in the sparsely distributed tree *Kalopanax pictus*. Journal of Plant Research 119(3):195-204.

Ghazoul J, McLeish M (2001) Reproductive ecology of tropical forest trees in logged and fragmented habitats in Thailand and Costa Rica. Plant Ecology 153 (1-2): 335-345.

Gleeson SK (1982) Heterodichogamy in walnuts: inheritance and stable ratios. Evolution 36:892-902.

Hille Ris Lambers J, Clark JS (2003) Effects of dispersal, shrubs, and density-dependent mortality on seed and seedling distributions in temperate forests. Canadian Journal of Forest Research 33:783-795.

House SM (1992) Population density and fruit set in three dioecious tree species in Australian tropical rain forest. Journal of Ecology 80:57-69.

Isagi Y. Tateno R, Matsuki Y, Hirao A, Watanabe S, Shibata M (2007) Genetic and reproductive consequences of forest fragmentation for populations of *Magnolia obovata*. Ecological Research 22:382-389.

Jong TJ de, Klinkhamer PGL (1989) Size-dependency of sex-allocation in hermaphroditic, monocarpic plants. Functional Ecology 3:201-206.

Kimura M, Seiwa K, Suyama Y, Ueno N (2003) Flowering system of heterodichogamous *Juglans ailanthifolia*. Plant Species Biology 18:75-84.

Klinkhamer PGL, Jong TJ de, Bruyn GJ de (1989) Plant size and pollinator visitation in *Cynoglossum officinale*. Oikos 54:201-204.

Kunin WE (1997) Population size and density effects in pollination: Pollinator foraging and plant reproductive success in experimental arrays of *Brassica kaber*. Journal of Ecology 85 (2): 225-234.

Lloyd DG, Webb CJ (1986) The avoidance of interference between the presentation of pollen and stigmas in anginosperms. I. Dichogamy. New Zealand Journal of Botany 24:135-162.

Matsui K (1991) Pollination ecology of four *Acer* species in Japan with special reference to bee pollinators. Plant Species Biology 6:117-120.

Ohlson M (1988) Size-dependent reproductive effort in three populations of *Saxifraga hirculus* in Sweden. Journal of Ecology 76(4):1007-1016.

Pendleton RL, Freeman DC, McArther ED, Sanderson SC (2000) Gender specialization in heterodichogamous *Grayia brandegei* (Chenopodiaceae): evidence for an alternative pathway to dioecy. American Journal of Botany 87(4):508-516.

Renner SS (2001) How common is heterodichogamy? Trends in Ecology and Evolution. 16(11):595-597.

Ritland K, Jain SK (1981) A model for the estimation of outcrossing rate and gene frequencies using *n* independent loci. Heredity, 47, 35-52.

Sasaki M (1999) Wonders of the Japanese Honeybee, Biology of Northernmost *Apis cerana*. (In Japanese) Kaiyusha Publishers, Tokyo pp10-11.

Sato T (2002) Phenology of sex expression and gender variation in a heterodichogamous maple, *Acer japonicum*. Ecology 83(5):1226-1238.

Shibata M, Nakashizuka T (1995) Seed and seedling demography of four co-occurring *Carpinus* species in a temperate deciduous forest. Ecology 76(4): 1099-1108.

Steffan-Dewenter I, Munzenberg U, and Tscharntke T (2001) Pollination, seed set and seed predation on a landscape scale. P. Roy. Soc. of Lond. B Bio. 268(1477):1685-90.

Steffan-Dewenter I, Munzenberg U, Burger C (2002) Scale-dependent effects of landscape context on three pollinator guilds. Ecology 83(5):1421-1432.

Sueyoshi M, Maeto K, Makihara H, Makino S, Iwai T (2003) Changes in dipteran assemblages with secondary succession of temperate deciduous forests following clear-cutting. (In Japanese with English summary) Bulletin of FFPRI 2(3):171-191.

Suzuki W (2002) Forest vegetation in and around Ogawa Forest Reserve in relation to human impact. In: Nakashizuka T and Matsumoto Y (eds), Ecological studies 158, Springer-Verlag,Tokyo pp. 27-40.

Visscher PK, Seeley DK (1982) Foraging strategy of honeybee colonies in a temperate deciduous forest. Ecology 63: 1790-1801.

Wagenius S (2006) Scale dependence of reproductive failure in fragmented *Echinacea* populations. Ecology 87(4): 931-941.

Wright SJ, Muller-Landau HC, Calderon O, Hernandes A (2005) Annual and spatial variation in seedfall and seedling recruitment in a neotropical forest. Ecology 86(4):848-860.

Table 1. Multiple regression analyses of factors affecting the fruiting process.

	Factors							
Demographic parameters	Distance from eighboring adults		Tree size		Sex morph		Regression model's	
	effect	Prob.	effect	Prob.	effect	Prob.	R^2	AIC
Seed mortality								
Pollination failure								
Immature seed fall	＋	0.014	ns	---	PG<PA	0.005	0.41	151.8
Empty seeds	＋	<.0001	ns	---	ns	---	0.26	437.4
Suffering predation	-	0.041	ns	---	ns	---	0.07	423.9
Decay	-	0.003	ns	---	ns	---	0.13	439.9
Seed maturation								
Soundness	ns	---	ns	---	ns	---	<.00	467.2
Seed kernel weight	(＋)	0.071	ns	---			0.56	91.8

DIS-RS and DBH are shown as an indicator of distance from conspecific adults and tree size, respectively.

Symbols of effect are as follows; ＋: a positive effect, -: a negative effect, (+): marginally significant positive effect, ns: no significant effect.

Fruit Utilization by Five Types of Understory Bird Assemblages in Sarawak, Malaysia

Tamaki Kamoi[1], Oswald Braken[2], Kuniyasu Momose[1]
[1] *Faculty of Agriculture, Ehime University, Japan*
[2] *Sarawak Forestry Corporation, Malaysia*

Introduction

In the Oriental Region, highly frugivorous bird species occur in 17 families (of 82), and some degree of frugivory has been reported for 50% of bird families (Corlett 1998). Many frugivorous birds feed on only a portion of the diversity of fleshy fruits produced in any habitat. Fruit selection presumably depends on the behavior, morphology, and nutritional requirements of birds, the abundance of alternative food resources, and fruits characteristics such as temporal availability, habitat, color, abundance, pulp-to-seed ratio and nutrient composition (Corlett 1998, Herrera 1998, Witmer 2001, Alcántara and Rey 2003).

Fruit size may be critical to selection. Sekercioglu (2006) argued that large-seeded plants depend on large frugivores (e.g., hornbills), which account for a relatively small part of the avian fauna, for seed dispersal. Since larger birds have larger gapes, they could potentially eat fruits with a wider range of seed size and a larger number of fruit species. However, it is unlikely that large birds eat small fruits in open spaces and the understory. Therefore, understory birds with gapes of different size should also play some roles in seed dispersal in open spaces and the understory.

The relative abundance of bird groups, based on gape width and frugivory level, was clearly different among forest types (Moran et al. 2004). Also, they discussed potential fruit utilization; however, it is essential to connect gape size with plant species that frugivorous birds actually eat for approach to ecological and conservational subjects. Also, previous studies have investigated how the distribution of understory frugivorous birds changed within a forest (Restrero et al. 1999; Pearman 2002). However, there has been little research that birds actually feed on how fruits of different plant life forms. By investigating it, the characteristic of fruits utilization of birds within a forest can be expressed.

The purpose of our study was to examine the relationship between smaller birds living in the understory and their fruit utilization in Sarawak, Malaysia. More specifically, we asked three questions: (1) How fruits do understory birds eat on plant life forms? (2) Do understory birds with larger gapes utilize a larger number of fruit species? (3) Do birds with similar gape size overlap about the fruit species they consume?

Methods

Study site

This study was conducted in Lambir Hills National Park (4˚12'N, 114˚02'E), Sarawak, Malaysia. The rain forest in this park is composed of primary mixed dipterocarp (Dipterocarpaceae) forest.

The relationships between birds and fruits

To clarify the fruit species eaten by understory birds, we conducted three investigations. We referred to

Robson (2000) for the identification of bird species in the field. If the captured bird did not appear in Robson, we referred to MacKinnon and Phillipps (1993).

Indirect observation We set sensor cameras near fruiting trees in the canopy and understory for one or more weeks from April 2005 to June 2006, except in November 2005 and from February to April 2006. The camera lens was pointed toward the fruits. Photographed birds were then identified.

Direct observation The birds visiting fruiting trees were observed using a field scope and binoculars from April 2005 to January 2006, except in August and November 2005. We recorded the species of birds that ate fruits. We intensively observed trees bearing many fruits or those growing in open spaces where the sensor cameras could not be set.

Captured birds and excreted seeds We caught birds with mist nets from March 2005 to June 2006, except in August and November 2005 and from February to April 2006. Two mist nets per day per site were set at a total of five points at the forest edge or in the understory of the forest interior from 07:00 to 17:00. We used four shelf-mist nets that were 6 or 12 m long and 2.5 m high and had a 24- or 36-mm mesh size. The captured birds were carefully removed from the nets and kept for 1 hour in cotton bags where they could not hurt themselves. After the hour, we measured their body mass, wing length, tail length, gape length, and gape width, and released all birds uninjured. We then recorded the number, weight, and size of seeds contained in the excrement found in the cotton bag. The seeds were identified by comparing them with seeds of fruiting plants that had been collected during the field study.

Analysis When excreted seeds could not be identified, the data were not included in the analysis. Birds seen feeding on fruits during direct and indirect observations were included in the analysis along with data from the captured birds.

We showed fruit utilization by the five bird assemblages based on taxonomy and gape width, which were observed feeding on fruits of 28 plant species. The plant species were classified into three groups in terms of life forms: pioneer plants (forest edge and gaps), understory plants (understory of the forest interior), and canopy plants (canopy). Climbing and epiphytic *Ficus* species were categorized as canopy plants.

Results

Of the 56 species (15 families) that we captured, 19 species (four families) excreted feces containing seeds (Table 1). Captured frugivorous species recorded were divided into five types based on taxonomy and gape width (Table 2).Of seven emerald doves (*Chalcophaps indica*), one excreted intact seeds of *Macaranga bancana* (Euphorbiaceae); however, three doves excreted destroyed seeds. For this reason, we considered that *C. indica* was a seed predator rather than a seed disperser, and *C. indica* was not included in further analysis. Also, a little spiderhunter (*Arachnothera longirostra*) excreted intact seeds, however; *A. longirostra* was not included in further analysis because the seeds were not identified. In contrast, yellow-vented bulbuls (*Pycnonotus goiavier*) and Cream-vented bulbuls (*Pycnonotus simplex*) did not excrete seeds. However, these two bulbuls were included in further analysis because it was recorded that they fed on some fruits in direct observation.

All five types of frugivorous species utilized pioneer plants (Fig. 1). Medium-gaped bulbuls fed on various fruit species (25 of the 28 available species). Moreover, bulbuls with small and medium gapes utilized all three types of habitat for feeding on fruits. Barbets and flowerpeckers did not feed on understory fruits, and sunbirds did not feed on canopy fruits.

Diets of bird types have no overlap in understory plants, except for the overlap between small-gaped and medium-gaped bulbuls (Fig. 2). Barbets fed on fruits of pioneer and canopy plants but did not utilize understory plants. Fruits of *Ficus* accounted for three of seven plants. Fruit diets of flowerpeckers were composed of pioneer plants and *Ficus*. The fruit diet of small-gaped bulbuls was completely included in that of medium-gaped bulbuls. The fruit consumed by sunbirds did not overlap with that of barbets or flowerpeckers. Sunbirds fed only on four fruit species, three of which were pioneer *Macaranga* trees.

Discussion

Many pioneer trees produce fruits frequently (Davies and Ashton 1999; Sakai et al. 1999; our observations) and attract avian dispersers. At our study site, all understory birds fed on fruits of pioneer plants. Pioneer plants might constantly support understory frugivorous birds. Also, bulbuls fed on a wide range of fruits (Figs. 1, 2). In particular, they fed on a larger number of understory fruit species than the other bird groups. Hence, bulbuls seem to be essential for the seed dispersal of understory trees.

Of the five bird types, it was expected that barbets, which have the largest gapes, could potentially eat the largest number of fruit species. However, medium-gaped bulbuls utilized a greater number of different types of fruit than barbets (Fig. 1). In the same family (Pycnonotidae), small-gaped bulbuls fed on fewer fruit species than medium-gaped bulbuls. There are two possible reasons why medium-gaped bulbuls utilized all kinds of fruits. First, Pycnonotidae birds may not be very selective in feeding on fruits and can therefore utilize a wide variety of fruits. Second, a medium-sized gape might enable them to feed on fruits having a wider range of seed size, making them the most adaptable frugivorous birds in the forest understory.

Both the taxonomy and gape width of sunbirds were similar to those of flowerpeckers (Table 2); however, the fruit species eaten by sunbirds did not completely overlap with those eaten by flowerpeckers (Fig. 2). In general, frugivores select fruits in terms of fruit color, fruit size, seed size, and nutrient composition of the fresh pulp (Stiles 1993; Bollen et al. 2004). Both sunbirds and flowerpeckers utilize fruits; however, sunbirds mainly feed on nectar and insects and flowerpeckers mainly feed on the fruits (Corlett 1998). Sugar preferences also differ between nectar- and fruit-eating birds (Lotz 2006), and fruits eaten by these two bird types might be distinguished by the nutrient composition of the pulp.

All understory birds fed on fruits of pioneer plants. Pioneer plants might constantly support understory frugivorous birds. Also, Taxonomy rather than gape size might explain fruit utilization of understory birds.

Acknowledgments

The authors thank Daisuke Fukuda and Shoko Sakai, Kyoto University, for their great help in getting the permits to research and catch the birds; Takahito Yamamoto, Ehime Pref. Science Museum, for teaching us to use mist nets and to handle captured birds and for giving useful advice; Yumi Aihara, Ehime University, for field assistance and advice; and Ikuo Ninomiya and his students, Ehime University, for their valuable advice. This research was carried out as a part of project #2 of the Research Institute of Humanity and Nature organized by Tohru Nakashizuka and Masahiro Ichikawa. We thank the organizers of these projects and their Malaysian counterparts, Lucy Chong, Sarawak Forest Corporation, and Josef J. Kendawang, Sarawak Forestry Department, as well as other project members.

References

Alcántara JM, Rey PJ (2003) Conflicting selection pressures on seed size: evolutionary ecology of fruit size in a bird-dispersed tree, *Olea europaea*. J. Evol. Biol. 16:1168–1176.

Bollen A, Van Elsacker L, Ganzhorn JU (2004) Relations between fruits and disperser assemblages in a Malagasy littoral forest: a community-level approach. J. Trop. Ecol. 20:599–612.

Corlett RT (1998) Frugivory and seed dispersal by vertebrates in the Oriental (Indmalayan) Region. Biol. Rev. 73:413–448.

Davies SJ, Ashton PS (1999) Phenology and fecundity in 11 sympatric pioneer species of *Macaranga* (Euphorbiacea) in Borneo. Am. J. Bot. 86:1786–1795.

Herrera CM (1998) Long-term dynamics of Mediterranean frugivorous birds and fleshy fruits: a 12-year study. Ecol. Monogr. 68:511–538.

Lotz CN (2006) Sugar preferences in nectar- and fruit-eating birds: behavioral patterns and physiological causes. BIOTROPICA 38:3–15.

MacKinnon J, Phillipps K (1993) A Field Guide to The Birds of Borneo, Sumatra, Java and Bali. Oxford University Press.

Moran C, Catterall CP, Green RJ, Olsen MF (2004) Functional variation among fruigivorous birds: implications for rainforest seed dispersal in a fragmented subtropical landscape. Oecologia 141:584–595.

Pearman PB (2002) The scale of community structure: habitat variation and avian guilds in tropical forest understory. Ecol. Monogr. 72:19–39.

Restrero C, Gomez N, Heredia S (1999) Anthropogenic edges, treefall gaps, and fruit-frugivore interactions in a neotropical montane forest. Ecology 80:668–685.

Robson C (2000) A Field Guide To The Birds of South-East Asia. New Holland Publishers (UK).

Sakai S, Momose K, Yumoto T, Nagamitsu T, Nagamasu H, Hamid AA, Nakashizuka T, Inoue T (1999) Plant reproductive phenology over four years including an episode of general flowering in a lowland dipterocarp forest, Sarawak, Malaysia. Am. J. Bot. 86:1414–1436.

Sekercioglu CH (2006) Increasing awareness of avian ecological function. Trends Ecol. Evol. 21:464–471.

Stiles EW (1993) The influence of pulp lipids on fruit preference by birds. Vegetatio 107/108:227–235.

Witmer MC (2001) Nutritional interactions and fruit removal: Cedar Waxwing consumption of *Viburnum opulus* fruits in spring. Ecology 82:3120–3130.

Table 1. List of captured birds, the number and the proportion of the bird excreting seeds.

Faimly	Species	Common name	The number of captured birds	The number of the bird excreting seeds	The proportion of the bird excreting seeds
Apodidae	*Collocalia esculenta*	Glossy Swiftlet	2	0	0.00
Columbidae	*Chalcophaps indica*	Emerald Dove	7	1	0.14
Alcedinidae	*Alcedo meninting*	Blue-eared Kingfisher	9	0	0.00
	Ceyx rufidorsa	Rufous-backed Kingfisher	3	0	0.00
Halcyonidae	*Lacedo pulchella*	Banded Kingfisher	1	0	0.00
Cuculidae	*Cuculus micropterus*	Indian Cuckoo	2	0	0.00
	Surniculus lugubris	Drongo Cuckoo	1	0	0.00
Megalaimidae	*Megalaima mystacophanos*	Red-throated Barbet	3	3	1.00
Picidae	*Blythipicus rubiginosus*	Maroon Woodpecker	1	0	0.00
	Meiglyptes tukki	Buff-necked Woodpecker	7	0	0.00
	Sasia abnormis	Rufous Piculet	6	0	0.00
Cisticolidae	*Prinia flaviventris*	Yellow-bellied Prinia	2	0	0.00
Corvidae	*Hypothymis azurea*	Black-naped Monarch	7	0	0.00
	Rhipidura javanica	Pied Fantail	1	0	0.00
	Rhipidura perlata	Spotted Fantail	1	0	0.00
	Philentoma pyrhopterum	Rufous-winged Philentoma	2	0	0.00
Eurylaimidae	*Cymbirhynchus macrorhyncho*	Black-and-red Broadbill	1	0	0.00
Muscicapidae	*Copsychus malabaricus*	White-rumped Shama	14	0	0.00
	Cyornis caerulatus	Large-billed Blue-Flycatcher	1	0	0.00
	Cyornis turcosus	Malaysian Blue-Flycatcher	2	0	0.00
	Cyornis spp.	Blue-Flycatcher	3	0	0.00
	Rhinomyias umbratilis	Grey-chested Jungle-Flycatche	2	0	0.00
Nectariniidae	*Dicaeum trigonostigma*	Orange-bellied Flowerpecker	1	0	0.00
	Prionochilus maculatus	Yellow-breasted Flowerpecker	11	9	0.82
	Prionochilus xanthopygius	Yellow-rumped Flowerpecker	5	3	0.60
	Aethopyga siparaja	Crimson Sunbird	3	0	0.00
	Anthreptes malacensis	Plain-throated Sunbird	2	1	0.50
	Anthreptes rhodolaem	Red-throated Sunbird	1	1	1.00
	Anthreptes simplex	Plain Sunbird	7	5	0.71
	Anthreptes spp.	Sunbird	8	4	0.50
	Anthreptes singalensis	Ruby-cheeked Sunbird	3	0	0.00
	Arachnothera longirostra	Little Spiderhunter	96	1	0.01
	Hypogramma hypogrammicum	Purple-naped Sunbird	13	7	0.54
	unidentified	Sunbird	1	0	0.00
Passeridae	*Lonchura fuscans*	Dusky Munia	36	0	0.00
Pycnonotidae	*Alophoixus bres*	Grey-cheeked Bulbul	6	5	0.83
	Alophoixus phaeocephalus	Yellow-bellied Bulbul	15	8	0.53
	Iole olivacea	Buff-vented Bulbul	1	1	1.00
	Pycnonotus atriceps	Black-headed Bulbul	24	19	0.79
	Pycnonotus brunneus	Red-eyed Bulbul	12	11	0.92
	Pycnonotus erythropthalmos	Spectacled Bulbul	25	22	0.88
	Pycnonotus eutilotus	Puff-backed Bulbul	13	11	0.85
	Pycnonotus goiavier	Yellow-vented Bulbul	1	0	0.00
	Pycnonotus plumosus	Olive-winged Bulbul	10	6	0.60
	Pycnonotus simplex	Cream-vented Bulbul	1	0	0.00
	Tricholestes criniger	Hairy-backed Bulbul	3	2	0.67
Sylviidae	*Orthotomus atrogularis*	Dark-necked Tailorbird	4	0	0.00
	Orthotomus sericeus	Rufous-tailed Tailorbird	10	0	0.00
	Alcippe brunneicauda	Brown Fulvetta	1	0	0.00
	Macronous ptilosus	Fluffy-backed Tit-Babbler	3	0	0.00
	Malacocincla malaccensis	Short-tailed Babbler	3	0	0.00
	Malacocincla sepiarium	Horsfield's Babbler	1	0	0.00
	Malacopteron affine	Sooty-capped Babbler	1	0	0.00
	Malacopteron cinereum	Scaly-crowned Babbler	2	0	0.00
	Pellorneum capistratum	Black-capped Babbler	1	0	0.00
	Stachyris erythroptera	Chestnut-winged Babbler	8	0	0.00
	Stachyris maculata	Chestnut-rumped Babbler	6	0	0.00
	Stachyris nigricollis	Black-throated Babbler	2	0	0.00
	Trichastoma rostratum	White-chested Babbler	1	0	0.00
Total			419	120	0.29

Table 2. List of the analyzed 19 bird species and thier body mass and gape width.

Bird type	Faimly	Tribe	Species	Body mass (g)	Gape width (mm)	Gape size class
Barbets	Megalaimidae		*Megalaima mystacophanos*	75.2	21.59	l
Flowerpeckers	Nectariniidae	Dicaeini	*Dicaeum trigonostigma*	6.4	5.99	s
			Prionochilus maculatus	8.6	6.74	s
			Prionochilus xanthopygius	7.7	6.37	s
Sunbirds	Nectariniidae	Nectariniini	*Anthreptes malacensis*	11.4	7.08	s
			Anthreptes rhodolaem	NA	7.28	s
			Anthreptes simplex	8.4	6.73	s
			Hypogramma hypogrammicum	11.9	8.67	s
Medium-gaped bulbuls	Pycnonotidae		*Alophoixus bres*	42.0	14.16	m
			Alophoixus phaeocephalus	31.4	12.58	m
			Iole olivacea	24.4	10.74	m
			Pycnonotus brunneus	25.6	10.66	m
			Pycnonotus eutilotus	35.8	12.50	m
			Pycnonotus goiavier	28.0	12.79	m
			Pycnonotus plumosus	30.3	11.75	m
Small-gaped bulbuls	Pycnonotidae		*Pycnonotus atriceps*	22.0	9.88	s
			Pycnonotus erythropthalmos	17.7	9.61	s
			Pycnonotus simplex	23.0	9.27	s
			Tricholestes criniger	16.2	9.37	s

NA: not available, s: small-gaped (<10 mm), m: medium-gaped (10-15 mm), l: large-gaped (>15 mm).

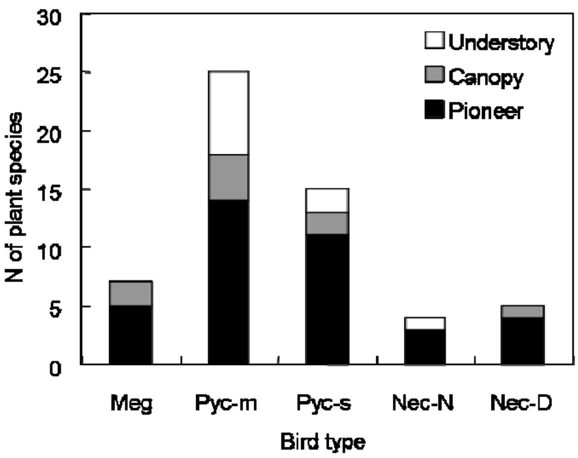
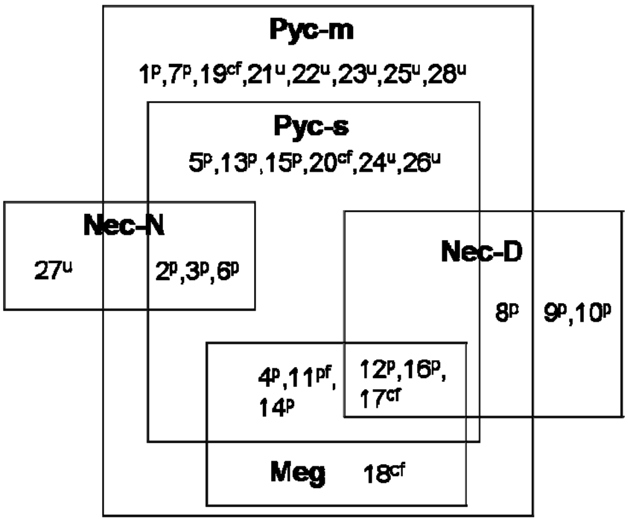


Fig. 1. The fruit utilization by five bird types: barbets (Meg), medium-gaped bulbuls (Pyc-m), small-gaped bulbuls (Pyc-s), sunbirds (Nec-N) and flowerpeckers (Nec-D).



1^{p}	*Dillenia suffruticosa*	(Dilleniaceae)
2^{p}	*Macaranga bancana*	(Euphorbiaceae)
3^{p}	*Macaranga beccariana*	(Euphorbiaceae)
4^{p}	*Macaranga gigantea*	(Euphorbiaceae)
5^{p}	*Macaranga hosei*	(Euphorbiaceae)
6^{p}	*Macaranga winkleri*	(Euphorbiaceae)
7^{p}	*Leea indica*	(Leeaceae)
8^{p}	*Melastoma malabathricum*	(Melastomatacea)
9^{p}	*Pternadra multiflora*	(Melastomatacea)
10^{p}	Melastomatacea sp.	(Melastomatacea)
11^{pf}	*Ficus fulva*	(Moraceae)
12^{p}	*Callicarpa pentandra*	(Verbenaceae)
13^{p}	*Callicarpa havilandii*	(Verbenaceae)
14^{p}	*Vitex pinnata*	(Verbenaceae)
15^{p}	Specimen NO.73	
16^{p}	Specimen NO.77	
17^{cf}	*Ficus acamptophylla*	(Moraceae)
18^{cf}	*Ficus stricta*	(Moraceae)
19^{cf}	*Ficus subgelderi*	(Moraceae)
20^{cf}	*Ficus sp1*	(Moraceae)
21^{u}	*Gomiphia serrata*	(Ochnaceae)
22^{u}	*Ixora woodii*	(Rubiaceae)
23^{u}	*Ixora sp2.*	(Rubiaceae)
24^{u}	*Lasianthus pilosus*	(Rubiaceae)
25^{u}	Specimen NO.38	
26^{u}	Specimen NO.44	
27^{u}	Specimen NO.76	
28^{u}	Specimen NO.88	

Fig. 2. The fruit diet information was classified into five bird types: barbets (Meg), medium-gaped bulbuls (Pyc-m), small-gaped bulbuls (Pyc-s), sunbirds (Nec-N) and flowerpeckers (Nec-D). In addition, the fruit species was classified into three groups: pioneer plants (p), understory plants (u), canopy plants (c). Climber/epiphyte plants of genus *Ficus* (f) were included in canopy plants.

Seed Dispersal by Mammals in Different Harvesting Intensities with Reduced-Impact and Conventional Logging in Sabah, Malaysia

Yoshihiro Nakashima[1, 2], **Hisashi Matsubayashi**[1, 3]
[1]*Center for Ecological Research, Kyoto University*
[2]*Present, Laboratory of Human Evolution Studies, Kyoto University*
[3]Present,Tokyo University of Agriculture

Introduction

Large mammals and birds are highly vulnerable to human disturbance such as hunting, habitat fragmentation and forest logging (Peres 2000), and their populations have rapidly diminished especially in Southeast Asia (Corlett 2002). Recent studies of fruit–frugivore interactions showed that large-seeded plants depend on large frugivores for seed dispersal (Corlett 1998; Kitamura et al. 2002). Large-seeded plants may thus be negatively affected by the decline of such animal populations. Urgent research is required into interactions between large-seeded fruits and frugivores where the latter still occur (Kitamura et al. 2002).

Reduced-impact logging is a set of guidelines to reduce the physical impacts on the ground, remaining standing trees, streams and ecosystem as a whole with the combination of a pre-harvest census, carefully controlled felling and skidding, lowered allowable cut and regulated machinery use (Putz and Pinard 1993). Previous studies showed that relative densities of frugivorous mammals such as orangutan and civets (Viverridae) were higher in reduced-impact logged forest than in conventional logged forest in Sabah, Malaysia (Ancrenaz et al. 2005; Onoguchi 2007). As a next research question, we need to know if and how the difference in the density of frugivorous mammals translates to seed dispersal.

To understand the fruit-frugivore interaction in different harvesting intensities between reduced-impact and conventional logging, we investigated the seed dispersal by mammals in both forests. We conducted (1) the identification of seed dispersers of genus *Durio* that is one of the largest-seeded groups in Southeast Asia, with direct observation and camera trap, and (2) the comparative study on seed dispersal by civets that is one of the most important seed dispersers in Southeast Asia with route census in reduced-impact logged forest and conventional logged forest.

Materials & Methods

Study site

Our study was conducted in Deramakot Forest Reserve (55,083ha), a reduced-impact logged forest and adjacent Tangkulap Forest Reserve (27,550ha), a conventionally logged forest, in Sabah, Malaysia. The climate is humid equatorial with a mean annual temperature of about 26℃. Mean annual rainfall is about 3,500 mm (Huth and Ditzer 2001). The major vegetation of Deramakot is a mixed dipterocarp forest dominated by the family Dipterocarpaceae, while that of Tangkulap is a forest dominated by pioneer species such as the genus *Macaranga* (Euphorbiaceae) (Seino et al. 2006). All of the large mammal species of

Sabah, including Asia elephants *Elephas maximus* (Linnaeus, 1758), orangutans *Pongo pygmaeus* (Hoppius, 1763) and sun bears *Helarctos malayanus* (Raffles, 1821), with the exception of the Sumatran rhinoceros *Dicerorhinus sumatrensis* (Fischer, 1814), inhabit these forests (Matsubayashi et al. 2006; Onoguchi 2007; Matsubayashi et al. in press).

Focal Durio species

The studied durian species were *Durio graveolens* Becc. and *D. zibethinus* Murray. Both species have the typical fruit structure of *Durio*, with large, tough husks and acute spines, containing arillate seeds, but the species vary in aril color, and the timing of dehiscence. *Durio graveolens* has a pale orange globose husk, 10.1 ± 1.4 cm long and 9.1 ± 1.2 cm wide (mean ± SD, n = 20), which completely splits into five valves on the tree (before the fruits fall). The seeds, 3.8 ± 0.5 cm long and 2.0 ± 0.1 cm wide (n = 30), are surrounded by red aril which is not sweet and has no odor. The number of seeds per fruit is 7.6 ± 2.2 (n = 20). In contrast, *D. zibethinus* has an ellipsoid green husk, 13.2 ± 3.1 cm long and 10.0 ± 3.3 cm wide (n = 20), which opens after the fruits have fallen. The seeds, 3.9 ± 0.3 cm long and 2.6 ± 0.2 cm wide (n = 30), are surrounded by white aril which is sweet in taste with a strong odor. The number of seeds per fruit is 5.5 ± 3.2 (n = 20).

Study sites are *ca* 30 km from the nearest village, and no fruits were removed by humans. In 2005, *Durio* bore many fruits from July to September in Deramakot. Observations were conducted at three *D. graveolens* trees (tree code: ET1, ET2, and ST3) and one *D. zibethinus* (K5). The *D. graveolens* trees were 223 ± 46 cm dbh, while the *D. zibethinus* was 345 cm. Our observations started before fruits were fully matured (developed full in fruit size with incomplete arillate seeds).

Camera trap and direct observation

When fruits were still immature (*i.e.*, intact on the trees for both species), both durian species were watched from a concealed position on the ground *ca* 20 m from the tree from 0530 h to 1830 h in almost all weather conditions except heavy rain (ET1: 28 July–7 August, ET2: 11–17 August, ST3: 5–12 September, K5: 3–16 August). When fruits matured (*i.e.*, those of *D. graveolens* retained on the tree, but those of *D. zibethinus* fallen), *D. graveolens* trees were observed directly from 0530 h to 0030 h (ET1: 8–10 August, ET2: 18–20 August, ST3: 13–19 September), and *D. zibethinus* was monitored with an automatic digital camera system (CAMEDIA digital camera X-350, OLYMPUS, and Magical Finger HAS-NF1, HOGA, Japan) on a 24-h basis (K5: 15–31 August). For the latter, we relocated all dropped fruits of *D. zibethinus* to the front of the automatic camera system.

In direct observation, we recorded the following: (1) visiting animal species; (2) length of time on tree; (3) the number of fruits consumed; and (4) the method of handling the seeds. We were unable to precisely count the number of fruits consumed, since fruits were concealed by leaves. Thus, the number of fruits consumed by each individual was estimated based on the number consumed during direct sightings divided by the proportion of the duration of direct sighting to the total duration of the visit. These points could be materialized for both species in most cases. Even when using an automatic camera, (3) and (4) were recorded by applying no photographic delay interval, which enabled us to use the camera like a video camera. Total fruit number was estimated from the total number of fallen husks with intact stalks during one season. This is likely to be an underestimate, because we may have overlooked fallen husks with intact stalks, and we observed that some animals consumed fruits after removal beyond the crown of a mother tree, so total fruit number here is approximate one. These observations were continued daily until all fruits which could be seen from the ground had been consumed. In total, we conducted 288 h direct observation on *D. graveolens* (ET1: 93 h, ET2: 65 h, ST3: 130 h), and 96 h direct observation and 384 h observation with the automatic camera on *D. zibethinus* (K5: 504 h).

Route census of seed dispersal

We conducted route census with a total of 30 km logging road, 17km in the reduced-impact logged forest for 52 days and 13km in the conventional logged forest for 23 days in 2004 and 2005 on foot and by motorbike during day. Dispersed seeds with feces of civets were photographed (Fig. 1), and the seeds were classified by aril color and shape. To identify seeds, we relied on Jaiwit P. and Gubilil M. who are the staff of Forest Research Centre, Sabah Forestry Department. We compared the frequency of the detection of the feces and number of the seeds species recorded in both of the forests.

Results

Seed dispersers of the Durio

Total fruit number was 99, 27, 252 and 58 at ET1, ET2, ST3 and K5, respectively. The results of our observations are shown in Table 1. We defined dispersal (D) as transporting seeds 20 m or more from the parent tree, neutral consumption (NC) as dropping intact seeds under the parent tree crown and predation (P) as destroying seeds.

During our observations of both species, orangutans consumed more fruits than any other consumers (ET1: $\chi^2 = 46.1$, df = 1, $P < 0.001$; ET2: $\chi^2 = 21.2$, df = 1, $P < 0.001$; ST3: $\chi^2 = 162$, df = 1, $P < 0.001$; K5: $\chi^2 = 29$, df = 1, $P < 0.001$; Table 1). When orangutans visited the trees, arillate seeds were still immature. They plucked fruits with hands and/or teeth, and easily tore apart husks with their hands and incisors. They picked up the arillate seeds, sucked aril, chewed the seed and spat out only the seed coats. At times, they made longer visits, making a bed on or near a fruiting tree and consuming fruits on two consecutive days. One male consumed 119 fruits during two days in one visit. In terms of function, they therefore appear to be the most important predators.

When *D. graveolens* matured, fruits dehisced on the trees, and they were consumed mainly by arboreal animals; Prevost’s squirrels *Callosciurus prevostii* (Desmarest, 1822), black hornbills *Anthracoceros malayanus* (Raffles, 1822), civets (Viverridae) and long-tailed macaques *Macaca fascicularis* (Raffles, 1821)(Table1). Black hornbills visited two trees (ET1, ST3), swallowed arillate seeds and did not regurgitate before leaving the tree, so they clearly dispersed seeds. The number of seeds handled by them (Table 1) was probably overestimated, especially at ST3, because they did not appear to eat fruits when perched in places that we were unable to observe. We directly observed hornbills eating only 7 seeds in 4 fruits. A civet visited one tree (ET1) during the night. The animal was concealed by leaves, so we were unable to identify it to species or how it handled the seeds. However, when we fed three captive common palm civets *Paradoxurus hermaphroditus* (Pallas, 1777) with *D. graveolens* fruits, they chewed and destroyed the seeds, and swallowed them (Y. Nakashima pers. obs.). In summary, *D. graveolens* seeds were predated mainly by orangutans and dispersed by black hornbills.

In contrast, *D. zibethinus* fruits were consumed once fallen to the ground, mainly by terrestrial animals (Table 1). Fruits remained unopened on the ground for some time. Before the fruits opened naturally, sun bears *Helarctos malayanus* visited and opened them with their clacks or teeth. The bears ate only arils and spat out intact seeds, so they are neutral consumers in our observations. Long-tailed giant rat *Leopoldamys sabanus* (Thomas, 1887), large tree shrew *Tupaia tana* (Raffles, 1821), horse-tailed squirrel *Sundasciurus hippurus* (Geoffroy, 1831), Malayan porcupine *Hystrix brachyura* (Linnaeus, 1758) and long-tailed porcupine *Trichys fasciculata* (Shaw, 1801) were observed to visit the seeds left by the sun bears. Long-tailed giant rats sometimes carried seeds in their mouths and these seeds were probably quickly predated. When we traced 200 seeds of *D. zibethinus* (and 100 *D. graveolens*) with a thread-marking method (Yasuda et al. 2000), most seeds were predated within 1 day, and we have no evidence that scatter-hoarded seeds grew to seedlings (Y. Nakashima, unpublished data). Long-tailed macaques visited when the fruits were already dehisced on the ground. They cleaned arils in front of the mouth and then dropped the seeds. They sometimes carried the fruits > 10 m by hand. We found seed and husk discarded 23 m from the mother tree (transporting the fruit the first 18 m). Thus, these macaques do transport seeds beyond the crown of a mother tree although this behavior may be rare. The *D. zibethinus* seeds were mainly predated by orangutans and dispersed by long-tailed macaques.

Seed dispersal by civets

Frequency of detection of the feces was greater in the reduced-impact logged forest (1.13feces/day; the total number of feces is 59; total census day is 52 days) than that of in the conventionally-logged forest (0.61feces/day; 14 feces and 23days). Table 2 shows the family of seeds and the probably number of species in each family. In total, 28 species of seeds were detected in two forests. Twenty-seven species were in reduced-impact logged forest, and nine species were in conventionally-logged forest. Eight of the nine species found in the conventionally-logged forest were also detected in the reduced-impact logged forest.

Discussion

Our results show that many fruits of the two durian (*Durio*) species are predated, especially by orangutans. The majority of seeds did not germinate due to high predation pressure by orangutans, despite high investment into extremely large fruits. Some studies reported that orangutans especially preferred the fruits of *Durio* (Rijksen 1978; Galdikas 1982, 1988; Leighton 1993). In areas inhabited by orangutans, *Durio* dispersal success is probably lower than that in uninhabited areas. Although we report a predominant role of orangutans as predators, we also observed them to discard > 1000 mature intact seeds under one *D. graveolens* tree (TL1). This suggests that seed handling strategy varies among individuals, or even between trees by the same individuals. Galdikas (1982) highlighted their role as dispersal agents after observing orangutans discard *D. oxleyanus* seeds up to 50 m away from a mother tree. However, at least in

Deramakot, this is unlikely to occur. We also found many discarded seed coats under the other 8 fruiting trees, suggesting that they usually destroy and eat seeds. We always found their beds on or near fruiting trees, indicating that they stayed for up to several days to consume fruits and did not transport the seeds beyond the crown of mother tree even when they spat out the seeds.

Ridley (1984) suggested that *Durio* species with small red arils are probably dispersed by hornbills, and *D. zibethinus* is dispersed by bears. Our observations support the former, but not the latter. When we fed two captive bears with *D. zibethinus* fruits at the Sepilok Orangutan Rehabilitation Centre in Sabah, they did not swallow seeds. However, one individual at Sandakan Crocodile Farm in Sabah swallowed and excreted the intact *D. zibethinus* seeds. These results suggest that seed handling strategies differ among and/or within individuals of bear. In the wild, the Asian elephant *E. maximus* can probably also disperse seeds of *D. zibethinus*. The intact seeds were observed in their feces in the natural habitat. (A. Ahmad who is a staff of Sabah Forestry Department, pers. comm.). In addition, Prevost's squirrel may carry the seeds of the two species at least as far as to the adjacent tree crowns, as was reported in Peninsular Malaysia (Becker et al. 1985), although we did not observe such a behavior.

Our results suggest that both *Durio* species studied face difficulties with seed dispersal due to high predation pressure, especially by orangutans, and limited occurrence of alternative dispersal agents. The low density of wild *Durio* may be in part caused by high predation pressure. Our study did not reveal the main seed dispersers of *D. zibethinus*, but it is clear that the two *Durio* species are dispersed by different animals; *D. graveolens* by hornbills and *D. zibethinus* by large terrestrial mammals such as elephant and bear. These animals have been reported to be negatively affected by habitat degradation including fragmentation and logging (Corlett 2002). Therefore, our results suggest that the loss of large animals due to habitat degradation affects the regeneration of both species of *Durio*.

The frequency of the occurrence of civet feces and the number of dispersed seeds tended to diminish in the conventionally logged forest than in the reduced-impact logged forest. The former may reflects the reduction of the density of civets in the conventionally-logged forest. Seven species of civets were recorded in Deramakot (Matsubayashi et al. 2006; Onoguchi 2007; Matsubayashi et al. in press). Although we could not identify the species of civet for each dispersed seed, much of the seeds might have been dispersed by common palm civet *P. hermaphroditus* and/or Malay civet *Viverra tangalunga* (Gray, 1832) because these two species were often observed on the road at night and the density (indexed by camera trap) of those species tended to decrease in the conventionally-logged forest (Onoguchi 2007). The latter may reflect the decrease of the food resources for civets in the conventionally-logged forest. For example, seeds of Sapotaceae (Fig.1; vernacular name, Nyatoh), a group of climax trees that are commercially harvested, were detected in the reduced-impact logged forest only. The seeds of Sapotaceae could not be detected in the conventionally-logged forest probably because Sapotaceae tree were heavily harvested.

Our results correspond with the previous comparative study on vegetation and mammal fauna in the two forests (Seino et al. 2006; Onoguchi 2007), and imply that heavier logging intensities cause the degradation of the ecological function of civets, which in turn may feed-back to the forest regeneration through reduced seed dispersal.

References

Ancrenaz M, Gimenez O, Ambu L, Ancrenaz K, Andau P, Goossens B, Payne J, Sawang A, Tuuga A, Lackman-Ancrenaz I (2005) Aerial surveys give new estimates for orangutans in Sabah, Malaysia. PLoS Biology, 3, 1-8.

Becker P, Leighton M, and Payne JB (1985) Why tropical squirrels carry seeds out of source crowns? Journal of Tropical Ecology 1: 183-186.

Corlett RT (1998) Frugivory and seed dispersal by vertebrates in the Oriental (Indomalayan) region. Biological Review 73: 413-448.

Corlett RT (2002) Frugivory and seed dispersal in degraded tropical East Asian landscapes. In D. Levey, W. Silva and M. Galetti. (Eds). Seed dispersal and frugivory: ecology, evolution and conservation. pp 451-465. CABI Publishing, New York, New York.

Galdikas BMF (1982) Orangutans as seed dispersers at Tanjung Puting, Central Kalimantan: Implications for conservation. In L. E. M. Boer (Ed.). The Orangutan. Its Biology and Conservation. pp 285-298. Boston Hingham, The Hague, The Netherlands.

Galdikas BMF (1988) Orangutan diet, range, and activity at Tanjung Puting, Central Borneo. International Journal of Primatology 9: 1-35.

Huth A and Ditzer T (2004) Long-term impacts of logging in a tropical rain forest – a simulation study. Forest Ecology and Management 142: 33-51.

Kitamura S, Yumoto T, Poonswad P, Chuailua P, Plongmai K, Maruhashi T, and Noma N (2002) Interactions between fleshy fruits and frugivores in a tropical seasonal forest in Thailand. Oecologia 133: 559-572.

Leighton M (1993) Modeling dietary selectively by Bornean orangutans: evidence for integration of multiple criteria in fruit selection. International Journal of Primatology 14: 257-313.

Matsubayashi H, Lagan P, Majalap N, Tangah J, Sukor JRA, and Kitayama K (2006) Diversity of mammalian species at natural licks in rain forest of Deramakot and their conservation. In: Lee YF et al. (eds.) Synergy between carbon management and biodiversity conservation in tropical rain forests, the proceedings of the 2nd workshop, 30 Nov.-1 Dec. 2005, Sandakan, Malaysia. DIWPA, Shiga, Japan.

Matsubayashi H, Lagan P, Majalap N, Tangah J, Sukor JRA, and Kitayama K (in press) Importance of natural licks for the mammals in Bornean inland tropical rain forests. Ecological Research. (DOI: 10.1007/s11284-006-0313-4)

Onoguchi G (2007) Can reduced-impact logging preserve mammals in tropical rainforests? -A comparative survey on mammalian fauna by camera-trapping in Sabah, Borneo- Graduation thesis, Kyoto University.

Peres CA (2000) Effects of subsistence hunting on vertebrate community structure in Amazonian forests. Conservation Biology 14: 240-253.

Ridley HN (1894) On the dispersal of seeds by mammals. Journal of the straits branch of the royal Asiatic society 25: 11-32.

Rijksen HD (1978) A Field Study on Sumatran Orangutans (*Pongo pygmaeus abelii* Lesson 1827): Ecology, Behavior and Conservation. pp 420. H. Veenman & Zonen B. V. Wageningen, The Netherlands.

Seino T, Takyu M, Aiba S, Kitayama K, and Ong RC (2006) Floristic composition, stand structure, and above-ground biomass of the tropical rain forests of Deramakot and Tangkulap Forest Reserve in Malaysia under different forest managements. In: Lee YF et al. (eds.) Synergy between carbon management and biodiversity conservation in tropical rain forests, the proceedings of the 2nd workshop, 30 Nov.-1 Dec. 2005, Sandakan, Malaysia. DIWPA, Shiga, Japan.

Yasuda M, Miura S, and Hussein NA (2000) Evidence for food hoarding behaviour in terrestrial rodents in Pasoh Forest Reserve, a Malaysian lowland rain forest. Journal of Tropical Forest Science 12: 164-173.

Table 1. The estimated numbers of the durian fruits consumed by each of visiting animals.

Tree species	Tree code	Animal species	Seed handling[1]	Unripe fruit		Ripe fruit	
				Visits (*N*)	Fruit consumed (*N*)	Visits (*N*)	Fruit consumed (*N*)
D. graveolens	ET1	*Pongo pygmaeus* (♀)	P	1	62	0	0
		Callosciurus prevostii	NC/P	3	0	4	3
		Macaca fascicularis (♂)	NC	0	0	1	3
		Viverridae sp.	-	0	0	1	?
	ET2	*P. pygmaeus* (♀)	P	1	24	0	0
		C. prevostii	NC/P	2	0	1	1
		Anthracoceros malayanus (♂)	D	0	0	1	0
	ST3	*P. pygmaeus* (♂)	P	2	206	0	0
		C. prevostii	NC/P	0	0	1	4
		A. malayanus (♂)	D	0	0	3	12
D. zibethinus	K5	*P. pygmaeus* (♀)	P	1	52	0	0
		C. prevostii	NC/P	2	1	0	0
		Helarctos malayanus	NC	0	0	7	16
		M. fascicularis (♂, ♀)	NC/D	0	0	1	9
		Leopoldamys sabanus	P	0	0	1	1

Tree codes correspond to main text.

[1] P = predation, NC = neutral consumption, D = dispersal

Table 2. Family of the seeds found in civet feces and the estimated number of species in each family in the reduced-impact logged forest and the conventionally-logged forest.

Census sites	Family	Detected no. of species (total)
Reduced-impact logged forest	Annonaceae	2
	Cucurbitaceae	1
	Leeaceae	1
	Meliaceae	3
	Passifloraceae	1
	Sapotaceae	1
	Solanaceae	1
	Theaceae	1
	Unidentified	16
		(27)
Conventionally-logged forest	Meliaceae	1
	Passifloraceae	1
	Rubiaceae	1
	Unidentified	6
		(9)

Figure 1. Dispersed seeds (Sapotaceae) in the feces of civets in the reduced-impact logged forest.

Effects of Past Forest Use on *Ficus* Fruiting Behavior in the Western Lowlands of Yakushima Island

Tatsuya Otani[1], Kanetani Sei-ichi[2]
[1]*Kyushu Research Center, Forestry and Forest Products Research Institute*
[2]*Forestry and Forest Products Research Institute*

Introduction

Strangling *Ficus* trees develop a huge crown and produce large amounts of fruit throughout the year. Thus, they can act as "keystone" species in tropical and subtropical forests (Lambert & Marshall 1991; Richards 1996). In the lowlands of Yakushima Island, Japan, located at the southern end of the warm temperate zone, *Ficus superba* var. *japonica* provides fruits for Japanese macaque and various bird species throughout the year (e.g., Hanya et al. 2003), indicating the importance of this species in plant–animal interactions and forest ecosystems. However, it seems that year-round fruiting is more vulnerable in the temperate zone, in which environmental conditions fluctuate seasonally, than in the tropical zone, which has constant high temperatures.

In addition to seasonal changes in environmental conditions, the warm-temperate forest of the western lowlands of Yakushima Island, which is a World Natural Heritage area, experienced human disturbances such as clear-cut logging in the early 1940s (see section 2.1.3.3. for details). Past human impacts can affect the current landscape and tree distribution over a time span of 100 years (Ohtani & Koike 2005). Intensive logging in western Yakushima Island may have also affected ecological functions such as fruit production and the spatial distribution of a keystone *Ficus* species within the forest. Thus, we examined the effects of past logging on *Ficus* fruiting behavior in relation to its distribution and genetic characteristics.

Materials & Methods

The study site of Kawahara was located in the western coastal area of Yakushima Island, southwestern Japan (30°20' N, 130°30' E). The annual mean temperature was 19.8°C, and the monthly mean temperature ranged from 10.6°C in January to 26.2°C in August. This site was covered by secondary warm-temperate broad-leaved forest with dominant species such as *Quercus salicina* (Fagaceae), *Castanopsis sieboldii* (Fagaceae), *Litsea acuminata* (Lauraceae), *Ardisia sieboldii* (Myrsinaceae), and *Distylium racemosum* (Hamamelidaceae). Six *Ficus* species occurred at the study site: two stranglers, *F. superba* var. *japonica* and *F. microcarpa*; a shrub, *F. erecta*; and three vines, *F. nipponica*, *F. thunbergii*, and *F. pumila*. The strangler *F. superba* var. *japonica* occurred frequently in the lowlands of Yakushima Island. The density of the strangler *F. microcarpa* was much lower than that of *F. superba* var. *japonica* at the study site; the island is the northern limit of *F. microcarpa* habitat. Thus, we examined the fruiting phenology of *F. superba* var. *japonica*. Hereafter, "*Ficus* tree" refers to *F. superba* var. *japonica*.

In the Kawahara area, *Ficus* trees inhabited two types of site: sites that were subjected to intensive or clear-cut logging in the early 1940s; and sites that experienced relatively light logging mainly for charcoal production in the 1940s–1950s. Some huge strangler *Ficus* trees were found in the sites used for charcoal production. In contrast, most *Ficus* trees in the clear-cut sites had relatively small crowns and grew on rocks; these trees seemed to have established after the clear-cut logging.

In total, 74 *Ficus* trees were surveyed using a GPS receiver (Pathfinder Pro XRS, Trimble, Tokyo) and their crown projection area was determined as an index of tree size. Fruiting behavior has been monitored at 3- to 4-week intervals (on average, 26.4 days per interval) since June 2003, and the amount and maturity of fruit is recorded at each census. The amount of fruit was ranked from 0 = no fruit to 8 = vast amount of fruit, indicating the relative fruit abundance within an individual tree. Here, we analyzed data from June 2003 to December 2006.

To examine the relationship between genetic characteristics and fruiting patterns of individual trees, we conducted microsatellite genotyping of the 74 *Ficus* trees monitored. Fresh leaf samples were obtained from each tree between June and August 2004, and DNA was extracted using a DNeasy Plant Mini Kit (QIAGEN). We used six microsatellite primers that were developed for other *Ficus* species: FinsA1, FinsH5, FinsJ10, FinsM5, FM4-70, and FS3-31 (Vignes et al., 2006; Zavodna et al., 2005). The loci were amplified in two multiplex polymerase chain reactions (PCRs), with each containing four and two primer pairs (FinsH5 / FinsM5 / FM4-70 / FS3-31 and FinsA1 / FinsJ10) and 10 ng of template DNA. PCR amplifications were performed using a GeneAmp PCR System 9700 (Applied Biosystems) with: initial denaturation at 95°C for 15 min; 25 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 90 s, and extension at 72°C for 60 s; and a final incubation at 60°C for 30 min and holding at 4°C. PCR products were denatured at 95°C for 3 min and electrophoresed along with the GENESCAN 400-HD ROX size standard on a 3100 Genetic Analyzer using GENESCAN analysis software and GENOTYPER software (Applied Biosystems).

Results & Discussion

Of the 74 *Ficus* trees monitored, four were of relatively small size did not produce any fruits during the study period and so were excluded from the analyses. K-means clustering classified the 70 *Ficus* trees into two groups according to the amount of fruit at each census. The mean amount of fruit for the first group (20 trees) increased periodically in winter (Fig. 1). In contrast the mean amount of fruit for the second group (50 trees) was consistently low, with no apparent seasonal changes. The 20 trees that bore abundant fruit in winter had larger crown projection areas than the remaining 50 trees (U-test, $z = 3.18$, $p = 0.001$; Fig.2). The crown projection area was positively correlated with the fruiting frequency per year ($n = 70$, $r = 0.52$, $p < 0.001$; Fig. 2). This suggests that larger trees have the potential for frequent fruiting within a year and heavy fruiting in winter, which increases their relative contribution to the year-round fruiting and the plant–animal interactions.

The *Ficus* trees in Kawahara were distributed mainly in areas that were not clear-cut logged in the early 1940s, although some relatively small trees inhabited prior intensively logged sites (e.g., Fig. 3, southwestern corner). *Ficus* trees with the two types of fruiting pattern, i.e., heavy winter fruiting or no apparent trend, were randomly dispersed (Fig. 3), indicating no spatial bias in the tree distribution corresponding to the type of fruiting pattern. However, some large *Ficus* trees with heavy winter fruiting were located exclusively in sites that had not been clear-cut in the early 1940s (Fig. 3).

Six microsatellite loci were consistently resolved in the *Ficus* trees. A total of 23 alleles were detected: two for each of FinsJ10, FinsM5, and FS3-31; five for FinsH5; and six for each of FinsA1 and FM4-70. Based on the allele types for these six microsatellite loci, we performed a principal components analysis with respect to fruiting pattern (Fig. 4). The eigenvalues of axes 1 and 2 were 5.27 and 3.82, respectively. *Ficus* trees classified into the two fruiting patterns were scattered randomly in the analysis (Fig. 4), suggesting that the genotypes determined using the six microsatellite loci had no relation to the fruiting behavior and that the fruiting pattern of individual trees was governed by other factors such as variation in solar insolation.

The lack of large *Ficus* trees in the prior clear-cut sites indicated that the sizes and populations of *Ficus* trees are still developing and recovering from human disturbances that occurred approximately 60 years ago. Given their potential for heavy fruiting in winter, large *Ficus* trees can play a much more important role than small trees. However, some large *Ficus* trees growing on rocks or steep slopes fell over, probably as a result of strong wind and their own weight, implying a tree size limitation for *Ficus* tree growing on rocks. Large-sized host trees such as *Persea thunbergii* and *D. racemosum* appear to be required for the development of large strangling *Ficus* trees. Thus, it may take strangling *Ficus* species longer to recover than the general forest structure.

In the lowlands of Yakushima Island, as well as in the coastal areas of southwestern Japan, forests have been exposed to various human disturbances, including conversion to other land uses. Protected areas in lowlands and coastal sites are required to conserve rich ecosystems, including *Ficus*–animal interactions, in the warm temperate zone.

References

Hanya, G., Noma, N. and Agetsuma, N. (2003) Altitudinal and seasonal variations in the diet of Japanese macaques in Yakushima. Primates 44: 51-59.

Lambert, F. R. and Marshall, A. G. (1991) Keystone characteristics of bird-dispersed *Ficus* in a Malaysian lowland rain forest. Journal of Ecology 79: 793-809.

Ohtani, S. and Koike, F. (2005) Implications of 19th century landscape patterns for the recovery of *Fagus crenata* forests. Applied Vegetation Science 8: 125-132.

Richards, P. W. (1996) The tropical rain forest—an ecological study. Second edition. Cambridge University Press.

Vignes, H., Hossaert-Mckey, M., Beaune, D., Anstett, A. C., Borges, M., Kjellberg, F. and Chevallier, H. (2006) Development and characterization of microsatellite markers for a monoecious *Ficus* species, *Ficus insipida* and cross-species amplification among different sections of *Ficus*. Molecular Ecology Notes 6: 792-795.

Zavodna, M., Arens, P., Van Dijk, P. J. and Vosman, B. (2005) Development and characterization of microsatellite markers for two dioecious *Ficus* species. Molecular Ecology Notes 5: 355-357.

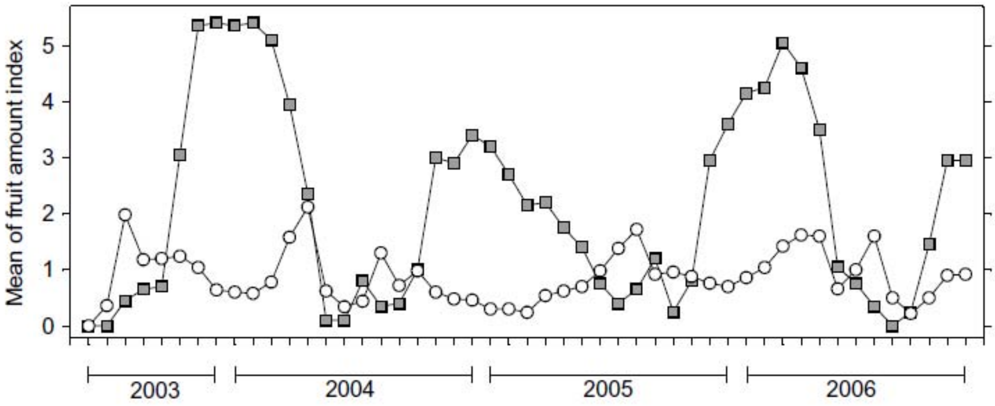


Fig. 1. Time series of the mean amount of fruit for two groups of *Ficus* classified using K-means clustering. One group (gray squares) tended to produce large amounts of fruit in winter; the other group (white circles) had no conspicuous seasonal trend.

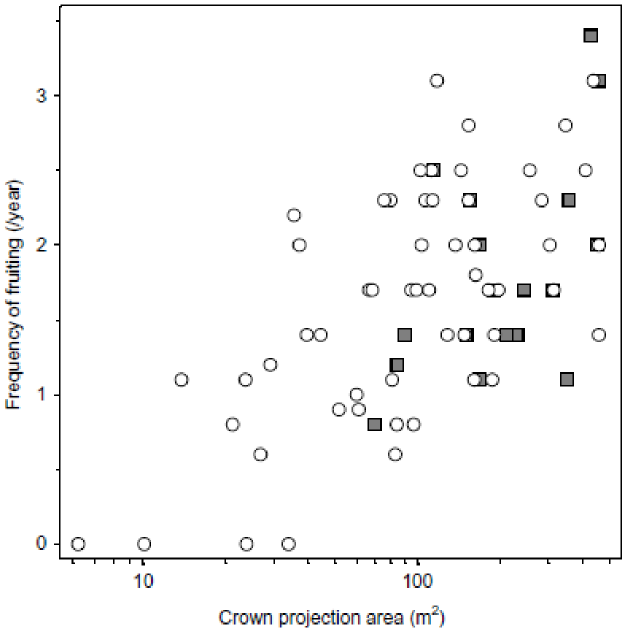


Fig. 2. The relationship between crown projection area and fruiting frequency per year of *Ficus superba* var. *japonica*. A significant positive correlation was detected (n = 70, r = 0.52, $p < 0.001$); four trees that did not produce fruit during the study period were excluded from the analysis.
See Fig. 1 legend for symbols.

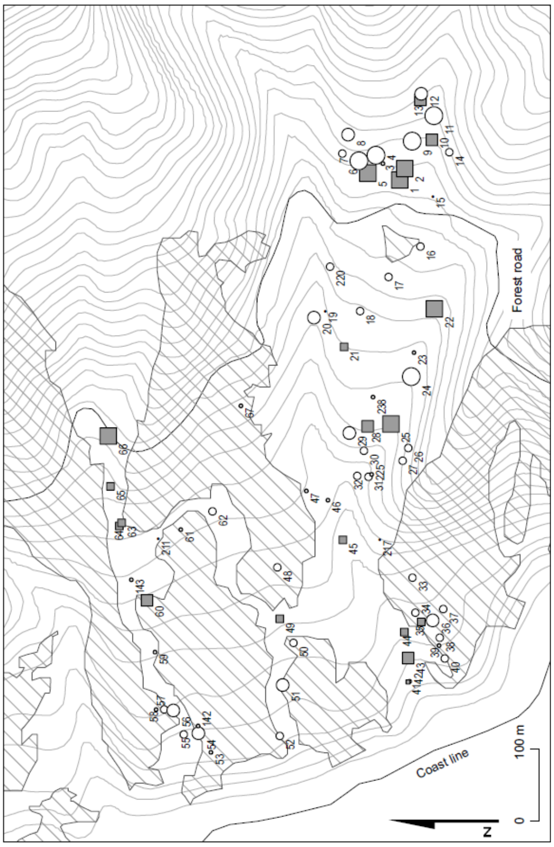


Fig. 3. The locations of *Ficus* trees classified into two groups based on fruiting pattern. See Fig. 1 legend for symbols. Symbol size represents relative tree size in four ranks. Shaded polygons indicate areas that were clear-cut in the early 1940s, derived from aerial photographs taken in 1947.

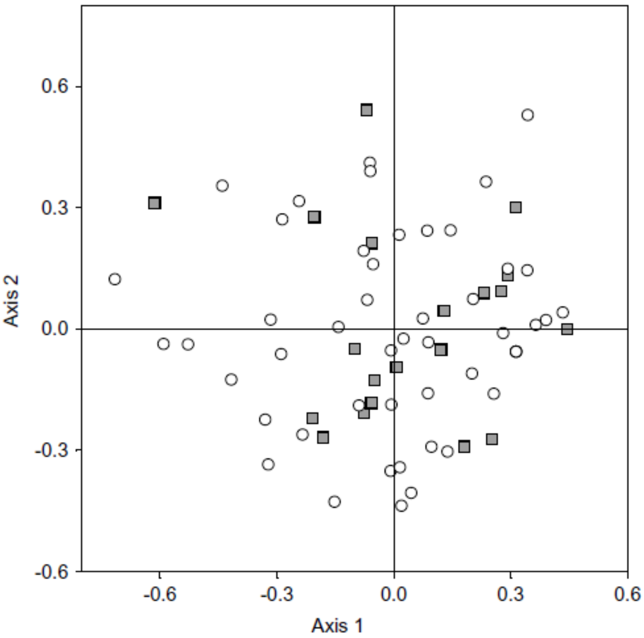


Fig. 4. Principal components analysis of the genotypes of 70 *Ficus* trees characterized using six microsatellite loci. See Fig. 1 legend for symbols.

Reduced Seed Dispersal Effectiveness of the Large-Seeded Tree *Myrica rubra* in the Absence of Japanese Macaque

Mari Terakawa[1], Yuji Isagi[2], Kiyoshi Matsui[3], Naohiko Noma[4], Satoshi Kikuchi[5], Seiichi Kanetani[5], Hiroshi Yoshimaru[5], Takakazu Yumoto[6]
[1]*Hirhoshima University*
[2]*Kyoto University*
[3]*Nara University of Education*
[4]*University of Shiga Prefecture*
[5]*Forestry and Forest Products Research Institute*
[6]*Research Institute for Humanity and Nature*

Introduction

Seed dispersal is a critical stage in a plant’s reproductive cycle for the purpose of establishing new populations. The process of seed dispersal not only enhances the fitness of the maternal plant (reviewed by Howe and Smallwood 1982; Willson and Traveset 2000), but also has a direct effect on the genetic structure of plant populations (Hamrick et al. 1993). Diaspores have many adaptations that enhance long-distance seed dispersal by using vectors such as wind, water, and animals. However, a large proportion of plants in most communities are dispersed by animals (Howe and Smallwood 1982). Therefore, seed dispersal by animals is considered to play an important role in the genetic structure of plant populations and the maintenance of biodiversity within forests.

Large frugivores are effective seed dispersers quantitatively, but they are extremely sensitive to habitat modifications caused by human activity. If plant species depend only on large frugivorous animals for seed dispersal, the disappearance of these animals because of human activity could lead to a failure in seed dispersal. A two-step approach may be used to assess the effect of human activity on seed dispersal: compare seed dispersal with and without the aid of large frugivores and determine what type of effect the extinction of these frugivores would have on the dispersal of plant populations.

We examined Japanese macaque (*Macaca fuscata*) as a large seed disperser of *Myrica rubra*, a large-seeded tree species. Potential seed dispersers of *M. rubra* may be limited because the fruit and seed sizes of *M. rubra* are large (fruit: 13.2 mm in diameter, seed: 7.7 mm in diameter) and small frugivores cannot swallow these seeds. To evaluate the effectiveness of seed dispersal by Japanese macaque, we compared the seed dispersal of *Myrica rubra* in two forests, one with and one without Japanese macaque. We also examined the seed dispersal of *M. rubra* using genetic analysis of seeds found in the feces of macaques.

Materials & Methods

Study site

The study sites were located in warm temperate evergreen forests on Yakushima Island (30°20' N, 130°30' E) and Tanegashima Island (30°43' N, 130°58' E), located in southwest Japan. Both islands shared similar low-altitude broad-leaved evergreen forests. On the western part of Yakushima Island, a subspecies of Japanese macaque (*Macaca fuscata yakui*) lived in social groups of 20–30 individuals in the wild, without provisioning, in a protected National Park. Furthermore, these macaques had been habituated to observers and their social and feeding ecology had been studied continuously since 1976 (Maruhashi 1980; Yumoto et al. 1998; Hanya 2003). In contrast, Japanese macaque is thought to have been extirpated from Tanegashima Island for approximately 80 years as a result of human activity.

Study species

Myrica rubra (Myricaceae) was a common dioecious evergreen tree on both islands. It produces a large amount of fleshy fruit from late May to late June, and the fruits are an important food source for frugivores, especially for Japanese macaque on Yakushima Island. Japanese macaque is a local seed disperser of *M. rubra*, along with some bird species. Because the Japanese macaque has cheek pouches (i.e., a pocket in the cheek that opens into the mouth), it can store various fruits and seeds and travel long distances before choosing to either spit out or swallow the seeds (Yumoto et al. 1998).

Comparison of fruit consumption with and without macaques

To collect information on frugivorous tree visitors, observations were carried out on one tree by a single observer on Yakushima Island (with macaques) and on Tanegashima Island (without macaques; total observation times: Yakushima Island 73 h 46 min, Tanegashima Island 63 h 44 min). Observations were conducted from 600 to 1800, except during rainy periods. All visitors to the focal trees and all species observed around the focal trees were recorded. The visiting and feeding times of each visitor were also noted. If direct observation was possible, the number of fruits consumed per minute was recorded. We calculated the number of fruits consumed by each visitor species per visit using data on the feeding period per visit and the number of fruits consumed per minute by each visitor species. Finally, we estimated the total number of consumed fruits on each tree per day using the average data of the number of fruits consumed by each visitor species per visit and the number of visitors per day.

Development of microsatellite markers for maternal analysis

To conduct a maternal analysis of seeds dispersed by macaques under natural conditions, we developed 13 microsatellite markers from a genomic library enriched for dinucleotide (CT) repeats in the dioecious evergreen tree *M. rubra* and screened loci from 32 adult trees (Terakawa et al. 2006).

Maternal analysis of seeds dispersed by macaques using microsatellite markers

To collect macaque feces, we followed a single habituated troop of Japanese macaque living in the evergreen forest on the western part of Yakushima Island from 600 to 1800 during three sessions in 2005: 25–30 May (late May), 4–6 June (early June), and 17–20 June (late June). This troop comprised 26 individuals: seven adult males, seven adult females, and 12 young individuals (E troop; Nishikawa, unpublished data).

We identified the maternal origins of dispersed seeds using the multilocus genotypes of 10 microsatellite loci of the seed endocarp. The genotype of endocarp tissue is identical to the genotype of the maternal tree because the endocarp is derived from the mother (Godoy and Jordano 2001). We determined the variation in maternal origin of dispersed seeds of *M. rubra* in feces of Japanese macaque during the fruiting period of *M. rubra* using the diversity and overlap index proposed by Grivet et al. (2005) as an index of the probability of maternal identity within and between gene pools. The diversity index of the maternal origin of dispersed seed ranges from 0 = highest diversity to 1 = lowest diversity. The overlap index of the maternal origin of dispersed seed ranges from 0 = lowest degree of overlap to 1 = highest degree of overlap. The inverse of the diversity index indicated the effective number of seed maternal origins; we calculated this for each period.

Results

Comparison of fruit consumption with and without macaques

We observed 25 species around the focal trees of *M. rubra* (Yakushima Island: Japanese macaque and 13 bird species; Tanegashima Island: 21 bird species). Six of the observed species were frugivores and five species were common to both islands. On Yakushima Island, the principal visitors of *M. rubra* were Japanese macaque and bulbul (*Hypsipetes amaurotis*; mean ± standard error number of visitors per day: macaque, 4.7 ± 1.4; bulbul, 3.2 ± 0.9; other birds, 0.3 ± 0.2). On Tanegashima Island, the principal visitor was bulbul (number of visitors per day: bulbul, 3.8 ± 0.9; other birds, 0.2 ± 0.1). There was no difference in the number of visits by bulbul between the two islands (*U*-test, $P > 0.05$).

The feeding time per visit of each visitor species differed significantly on Yakushima Island (mean ± standard error minutes per visit: macaque, 10.8 ± 1.0; bulbul, 0.8 ± 0.1; *U*-test, $P < 0.001$). However, there was no difference in the feeding time per visit for bulbul between the two islands (0.5 ± 0.1 min per visit; *U*-test, $P > 0.05$). The number of fruits consumed per minute by the macaques (14.0 ± 2.4) was greater than that by bulbul (5.5 ± 1.3). The number of fruits consumed per visit by the macaques was more than 30 times that by bulbul (Yakushima Island: macaque, 151.9 ± 13.8; bulbul, 4.3 ± 0.6; Tanegashima Island: bulbul, 3.0 ± 0.6). In summary, the number of fruits consumed per day per tree on Yakushima Island (all, 730.7; macaque, 716.9; bulbul, 13.8) was greater than on Tanegashima Island (all, 11.4; bulbul, 11.4).

Development of microsatellite markers for maternal analysis

The number of alleles of the microsatellite markers ranged from 2 to 14, and the expected heterozygosity ranged from 0.324 to 0.884 (Table 1). The total paternity exclusionary power when the mother was known was 0.99965. We tested linkage disequilibrium (LD) using Genepop version 3.4 software (Raymond and Rousset 1995). Three pairs of loci (my0043 and my0186, my0472 and my0792, and my0472 and my0793) showed significant LD in 78 pairwise tests with 13 loci ($P < 0.05$). These loci allow mating system and parentage analyses to be carried out for *M. rubra*, thereby supporting studies of *M. rubra* seed dispersal by macaques.

Genetic analysis of seed dispersal by macaques

We collected 89 feces in total: 19 in late May, 20 in early June, and 50 in late June. For all periods, the average number of seeds per feces was 8.56 (± 1.11 SE) with a range of 0 to 48. This number increased slightly between late May (9.26 ± 2.22 SE) and early June (15.95 ± 3.25 SE) and decreased significantly from early June to late June (5.34 ± 0.96). These changes in the number of seeds per feces in relation to the study period were statistically significant (Kruskall Wallis test: $H = 12.248$, $P = 0.002$), with a significant difference between early June and late June (Steel-Dwass test: $t = 3.251$, $P = 0.003$).

After genotyping, we identified 111 different maternal genotypes from 360 seeds in 41 feces (late May: 28 mothers from 106 seeds in 13 feces; early June: 52 mothers from 168 seeds in 11 feces; late June: 44 mothers from 86 seeds in 17 feces). The average number of different maternal genotypes per feces was 3.85 (± 0.40 SE), with a range of 2 to 11. Thus, macaques can disperse seeds from several different mother trees at a time (Fig. 1).

The average diversity of seed maternal origin per feces was very high (0.298) throughout the study period and increased from late May (0.425) to late June (0.239; 0.249 for early June). The total effective number of seed maternal origins in each period increased from late May (8.083) to late June (28.667).

Twenty-four maternal genotypes were shared between 92 pairs of feces of the 820 possible pairs (Fig. 1). Therefore, the seed maternal origin overlap among feces was very low (0.017). The seed maternal origin overlap among feces in each period decreased from late May (0.080) to late June (0.008).

Discussion

Comparison of fruit consumption with and without macaques

M. rubra seeds were dispersed by macaques and bulbul, and the disappearance of macaques could lead to a decrease in the number of seeds dispersed from mother trees. We observed a white-eye (*Zosterops japonicus*, gape size: 6.1 mm; Noma and Yumoto 1997) swallowing one red fruit of *M. rubra*. Bulbul and white-eye typically eat fruits, but capture insects to feed their offspring in the breading season. It is difficult for frugivorous birds on Tanegashima Island to fill the role in seed dispersal that macaques fill on Yakushima Island. This suggests that the loss of macaques may significantly affect seed dispersal, producing a gap that other frugivores may not be able to fill. Furthermore, the loss of seed dispersers as a result of human activity may affect tree species that produce sap fruits.

Seed dispersal of M. rubra by macaques on Yakushima Island

Macaques could disperse seeds from several maternal origins at the same time. Considering that the diversity of the maternal origin of *M. rubra* seed within feces was very high, macaques might efficiently enhance gene flow among local patches and populations of *M. rubra* and favor the settlement and growth populations with high genetic diversity. Seed dispersal is a critical process affecting genetic structure in plant populations (Hamrick et al. 1993). However, the seed dispersal range would still be limited by the macaque's home range; seed dispersal by macaques may play an important role the population genetic diversity of *M. rubra*.

Our results suggest that the feeding behavior of macaques determines the seed dispersal pattern of *M. rubra* on Yakushima Island. The number of seeds and maternal diversity of seeds within feces, and the maternal overlap of seeds among feces were different in each sampling period. Macaques changed their feeding behavior during the fruiting of *M. rubra* to conform to food availability (Agetsuma and Noma 1995).

References

Agetsuma N, Noma N (1995) Rapid shifting of foraging pattern by Yakushima macaques (*Macaca fuscata yakui*) as a reaction to heavy fruiting of *Myrica rubra*. Int J Primatol 16: 247–260.

Godoy JA, Jordano P (2001) Seed dispersal by animals: exact identification of source trees with endocarp DNA microsatellites. Mol Ecol 10: 2275–2283.

Grivet D, Smouse PE, Sork VL (2005) A novel approach to an old problem: tracking dispersed seeds. Mol Ecol 14: 3585–3595.

Hamrick JL, Murawski DA, Nason JD (1993) The influence of seed dispersal mechanisms on the genetic structure of tropical tree populations. Vegetatio 107/108: 281-297.

Hanya G (2003) Age differences in food intake and dietary selection of wild male Japanese macaques. Primates 44: 333–339.

Howe HF, Smallwood J (1982) Ecology of seed dispersal. Ann Rev Ecol Syst 13: 201–228.

Maruhashi T (1980) Feeding behavior and diet of the Japanese monkey (*Macaca fuscata yakui*) on Yakushima Island, Japan. Primates 21: 141–160.

Noma N, Yumoto T (1997) Fruiting phenology of animal-dispersed plants in response to winter migration of frugivores in a warm temperate forest on Yakushima Island, Japan. Ecol Res 12: 119–129.

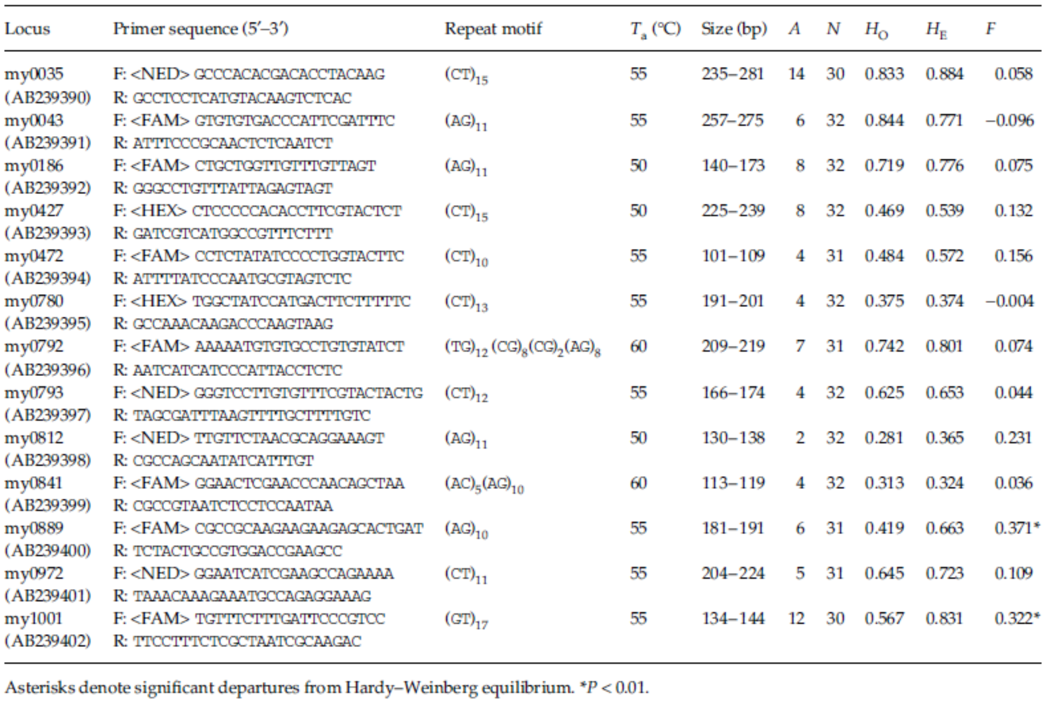
Raymond M, Rousset F (1995) Genepop (version 1.2): population genetics software for exact tests and ecumenicism. J Heredity 86: 248–249.

Terakawa M, Kikuchi S, Kanetani S, Matsui K, Yumoto T, Yoshimaru H (2006) Characterization of 13 polymorphic microsatellite loci for an evergreen tree, *Myrica rubra*. Mol Ecol Notes 6: 709–711.

Willson MF, Traveset A (2000) The ecology of seed dispersal. pp. 85–110. In: Seeds: The ecology of regeneration in plant communities. 2nd. Edition. M Fenner (ed.). CAB International, Wallingford, UK. [Reprint]

Yumoto T, Noma N, Maruhashi T (1998) Cheek-pouch dispersal of seeds by Japanese monkeys (*Macaca fuscata yakui*) on Yakushima Island, Japan. Primates 39: 325–338.

Table 1. Microsatellite loci for *Myrica rubra*, including primer sequences, repeat motifs, annealing temperatures (*T*a), size ranges (bp), number of alleles detected (*A*), number of genotyped individuals (*N*), observed heterozygosity (*H*O), expected heterozygosity (*H*E) and fixation index (*F*). DNA Database of Japan accession numbers are listed in parentheses under each locus.



Locus	Primer sequence (5′–3′)	Repeat motif	T_a (°C)	Size (bp)	*A*	*N*	H_O	H_E	*F*
my0035 (AB239390)	F: <NED> GCCCACACGACACCTACAAG R: GCCTCCTCATGTACAAGTCTCAC	$(CT)_{15}$	55	235–281	14	30	0.833	0.884	0.058
my0043 (AB239391)	F: <FAM> GTGTGTGACCCATTCGATTTC R: ATTTCCCGCAACTCTCAATCT	$(AG)_{11}$	55	257–275	6	32	0.844	0.771	−0.096
my0186 (AB239392)	F: <FAM> CTGCTGGTTGTTTGTTAGT R: GGGCCTGTTTATTAGAGTAGT	$(AG)_{11}$	50	140–173	8	32	0.719	0.776	0.075
my0427 (AB239393)	F: <HEX> CTCCCCCACACCTTCGTACTCT R: GATCGTCATGGCCGTTTCTTT	$(CT)_{15}$	50	225–239	8	32	0.469	0.539	0.132
my0472 (AB239394)	F: <FAM> CCTCTATATCCCCTGGTACTTC R: ATTTTATCCCAATGCGTAGTCTC	$(CT)_{10}$	55	101–109	4	31	0.484	0.572	0.156
my0780 (AB239395)	F: <HEX> TGGCTATCCATGACTTCTTTTTC R: GCCAAACAAGACCCAAGTAAG	$(CT)_{13}$	55	191–201	4	32	0.375	0.374	−0.004
my0792 (AB239396)	F: <FAM> AAAAATGTGTGCCTGTGTATCT R: AATCATCATCCCATTACCTCTC	$(TG)_{12}$ $(CG)_8(CG)_2(AG)_8$	60	209–219	7	31	0.742	0.801	0.074
my0793 (AB239397)	F: <NED> GGGTCCTTGTGTTTCGTACTACTG R: TAGCGATTTAAGTTTTGCTTTTGTC	$(CT)_{12}$	55	166–174	4	32	0.625	0.653	0.044
my0812 (AB239398)	F: <NED> TTGTTCTAACGCAGGAAAGT R: CGCCAGCAATATCATTTGT	$(AG)_{11}$	50	130–138	2	32	0.281	0.365	0.231
my0841 (AB239399)	F: <FAM> GGAACTCGAACCCAACAGCTAA R: CGCCGTAATCTCCTCCAATAA	$(AC)_5(AG)_{10}$	60	113–119	4	32	0.313	0.324	0.036
my0889 (AB239400)	F: <FAM> CGCCGCAAGAAGAAGAGCACTGAT R: TCTACTGCCGTGGACCGAAGCC	$(AG)_{10}$	55	181–191	6	31	0.419	0.663	0.371*
my0972 (AB239401)	F: <NED> GGAATCATCGAAGCCAGAAAA R: TAAACAAAGAAATGCCAGAGGAAAG	$(CT)_{11}$	55	204–224	5	31	0.645	0.723	0.109
my1001 (AB239402)	F: <FAM> TGTTTCTTTGATTCCCGTCC R: TTCCTTTCTCGCTAATCGCAAGAC	$(GT)_{17}$	55	134–144	12	30	0.567	0.831	0.322*

Asterisks denote significant departures from Hardy–Weinberg equilibrium. *$P < 0.01$.

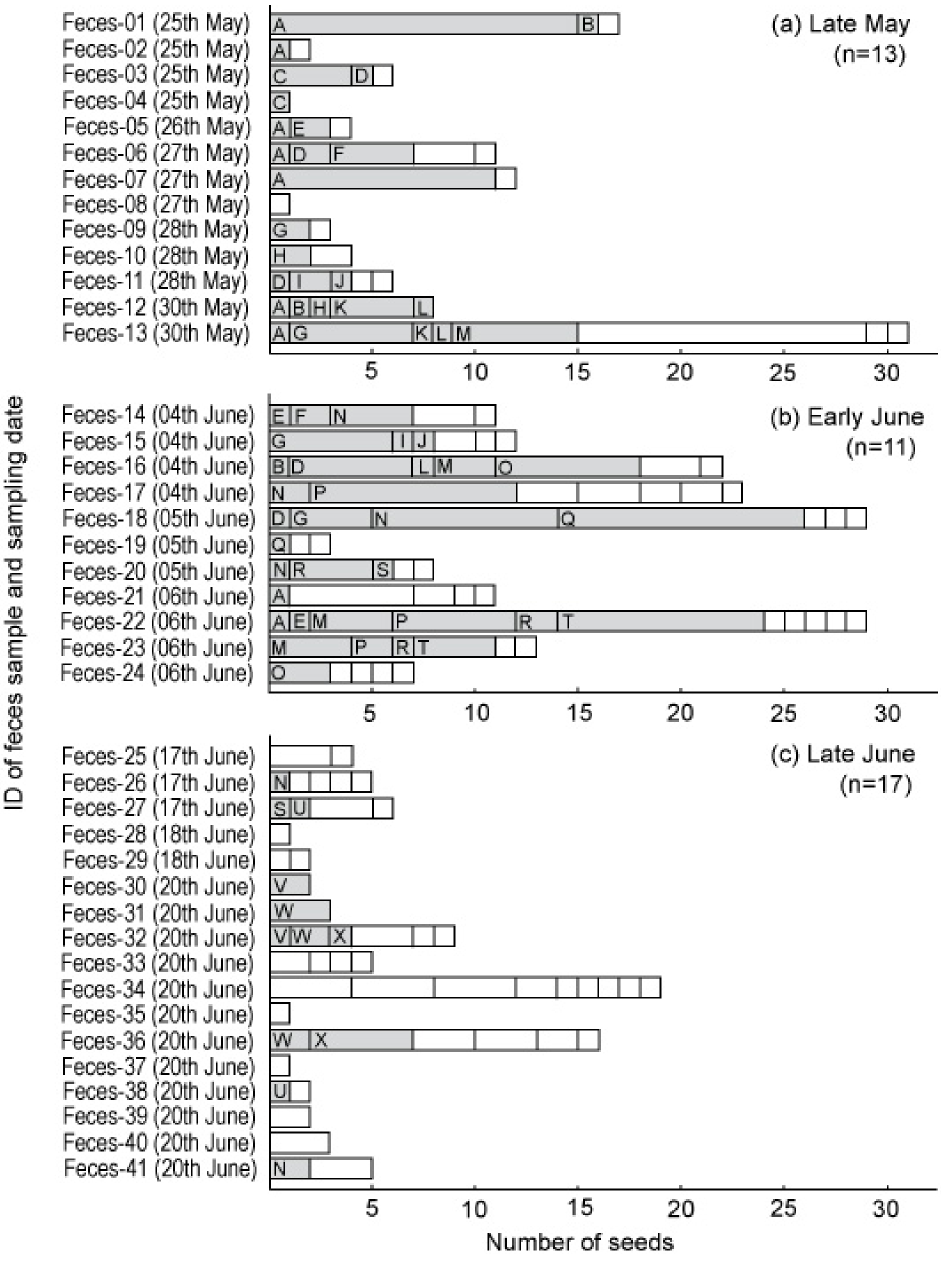


Figure 1. Number and maternal origin of seeds within feces sampled in (a) late May, (b) early June, and (c) late June. The same maternal origin of seeds found in two or more feces samples are indicated by a letter (A to X) and gray shading; n indicates the number of feces samples.

Pre-Dispersal Seed Predation of *Myrica rubra* on Yakushima Island

Mariko Fujita[1], Kiyoshi Matsui[2], Mari Terakawa[3], Furumi Komai[4], Takakazu Yumoto[5]
[1]*Kobe University*
[2]*Nara University of Education*
[3]*Hiroshima University*
[4]*Osaka University of Arts*
[5]*Research Institute for Humanity and Nature*

Introduction

Myrica rubra Sieb. et Zucc. (Myricaceae) is a common evergreen tree on Yakushima Island and produces a large amount of fleshy fruit. The fruit is an important food source for frugivores, especially for Yakushima macaques (*Macaca fuscata yakui*). However, *M. rubra* fruit production fluctuates greatly from year to year, and this annual change in crop size can affect the behavior of frugivores.

The predator satiation hypothesis was proposed by Janzen (1971) as one of ultimate causes of mast fruiting. Pre-dispersal seed predation has substantial negative effects on the relative reproductive success of individuals by limiting the number of viable seeds (Ida et al. 2004; Nakagawa et al. 2005; Sun et al. 2004). According to this hypothesis, many plants escape seed predators in mast years by controlling the number of predators in poor fruiting years via predator starvation. However, it is not known whether *M. rubra* suffers from seed predation and whether predator satiation reduces the proportion of seeds attacked.

We collected living and aborted fruit to identify major seed predators and to determine the proportion of fruit that was attacked by insects. In addition, we described the pre-dispersal survivorship patterns of fruit in 2006. We addressed the following questions. Who is the major seed predator of *M. rubra*? When does seed predation occur most heavily? Are there differences between the proportions of attacked living and attacked aborted fruit?

Materials & Methods

Study species

M. rubra is a dioecious evergreen tree that grows to approximately 15 m in height. On Yakushima Island, flowering starts from mid-March to early-April, and fleshy fruit is produced from late May to June. Each fruit has one seed. The fruit are an important food source for Japanese macaques as well as some bird species such as Japanese bulbuls.

Study site

The study site was located in a warm-temperate, evergreen, broad-leaved forest on Yakushima Island, which is located 70 km south of Kyushu, Japan. The canopy mainly consisted of Fagaceae, Hamamelidaceae, Myrsinaceae, and Lauraceae (Agetsuma 1995). The mean annual temperature was 21°C, and the annual rainfall was 2600 mm (Tagawa 1980).

In a 4-ha plot of a secondary stand (30°22’ 01.26” N, 130°23’ 08.23” E; 120 m above sea level), we deployed three seed traps (each covered a 0.5 m^2 area) below each of five female trees (one was added in 2006 for six trees) during the fruiting period. The seed traps were made of nylon cloth and were placed 1 m above the ground. To observe patterns of fruiting phenology and fruit predation, we placed a ladder at three of the six female trees in 2006 to access the crown.

Patterns of seed survivorship

To describe the pre-dispersal survivorship patterns of fruit, we marked 30 branches of each of three *M. rubra* trees and counted the numbers of surviving fruit > 1 mm in diameter on 8 April, 15 May, 4 June, and 15 June in 2006. Two of the three female trees were still flowering on 8 April; therefore, we estimated the numbers of fruit by multiplying the number of infructescences by the number of fruit per infructescence determined from observations of the other individual.

Seed predators and patterns of seed predation

Aborted fruit

To monitor the patterns of seed fate and predation, all of the fruit that fell into seed traps was collected weekly from the end of April to the end of June. We extracted and counted the number of fruit > 1 mm in diameter. Some of the fruit was measured: 10 fruit from each individual in 2005; 90 fruit from each individual in 2006. We ignored dropped flowers.

For each collection, we stored a maximum of 300 fruit per tree in 2005 and 30 fruit per tree in 2006 in plastic containers (280 cm^3) and allowed any insect larvae within the fruit to emerge. We placed up to 10 fruit in each container, along with a piece of moist cotton to prevent desiccation, and observed them for 3 months. The containers were checked every two days; if insects had emerged, they were collected, killed by freezing, and mounted on pins for later identification.

After 3 months, we dissected all of the fruit stored in the containers and classified each fruit as attacked or non-attacked. Fruit that was attacked by insects had larvae, feces, and/or a hole through which the insects had escaped from inside the fruit. We considered fruit that had these marks as attacked and that without these marks as non-attacked. In 2006, we also dissected 60 fruit from each female tree soon after collection from the seed traps for comparison with the proportion of surviving fruit that was also attacked by insects.

Living fruit

Throughout the fruiting period, 50 fruit per female tree were sampled from the canopy of three female trees using a ladder on 8 April, 15 May, 4 June, and 15 June in 2006. Upon collection, the diameter of each fruit was measured, and 10 fruit from each female tree were stored in containers in the same way as for aborted fruit. The remaining fruit was dissected and classified as attacked or non-attacked.

Data analysis

The proportion of attacked fruit was calculated by pooling the data for all female trees. For aborted fruit, the difference in the proportion of attacked fruit between 2005 and 2006 was examined using a chi-square test. To compare living and aborted fruit in 2006, we calculated the proportions of attacked fruit based on data from the fruit that was dissected upon collection, and the difference was examined using a chi-square test.

Results

Patterns of seed survivorship

Fruit mortality was highest during the first month after flowering. In all individuals, the numbers of living fruit on branches decreased rapidly from mid-April to mid-May and moderately from late May to mid-June (Fig. 1). By late July, no fruit remained on the branches.

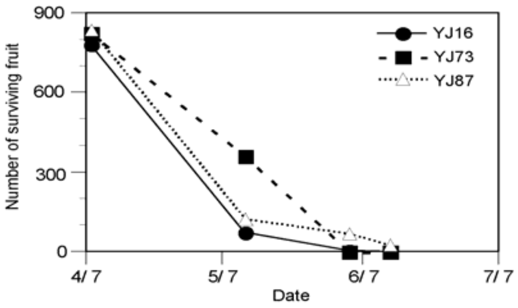


Fig. 1. Survivorship curves of fruit remaining on the branches of three female *Myrica rubra* trees. Symbols (●, ■ and △) indicate the total numbers of surviving fruit on 30 branches of each female tree.

Seed predators

In 2005, 598 individuals of *Thiotricha pancratiastis* Meyrick (Gelechiidae), two individuals of *Neoblastobasis spiniharpella* Kuznetzov & Sinev (Gelechiidae), and 109 individuals of parasitic wasp comprising seven species of Braconidae, two species of Ichneumonidae, and one species of Elasmidae emerged from 6188 stored fruit that were collected from seed traps. In 2006, 143 individuals of *T. pancratiastis* and 10 individuals of parasitic wasp emerged from 1090 stored fruit that were collected from seed traps. One individual of *T. pancratiastis* emerged from the stored fruit that was sampled directly from the branches (Fig. 2, Table 1).

Table 1. The number of insects that emerged from 6188 and 1090 aborted fruit in 2005 and 2006, respectively.

	Thiotricha pancratiastis	*Neoblastobasis spiniharpella*
2005	598	2
2006	143	0

Fate of fruit and predation

The total number of aborted fruit varied annually, at 10584 in 2005 and 6122 in 2006, and peaked at the early phase of development before fruit maturation (Fig. 3). In 2005, the peak was very clear; approximately 40% of aborted fruit fell during a single week from 30 April to 7 May. The mean ± standard deviation fruit size during this term was 7.7 ± 2.8 mm. Ripe fruit typically reached between 15 and 20 mm in diameter. It was clear that many immature fruit were aborted during this period. In 2006, 64% of aborted fruit dropped during the first month after flowering (Fig. 3). Insects attacked fruit intensively during the primary stage of fruit development. The proportion fruit attacked by insects reached as high as 64.9% between 7 and 14 May (Fig. 4). At other times, the attack rate was relatively low.

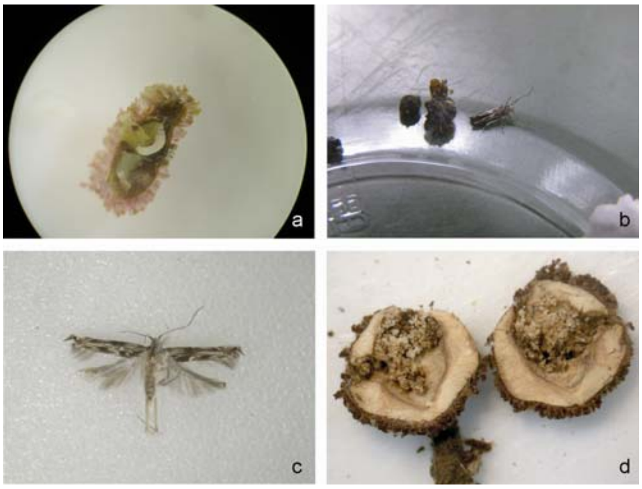


Fig. 2. Photographs of *Thiotricha pancratiastis* Meyrick (Gelechiidae) and attacked fruit. (a) Fruit infested by a larva. (b) *T. pancratiastis* adult just after emergence. (c) *T. pancratiastis* adult. (d) Attacked fruit containing insect feces.

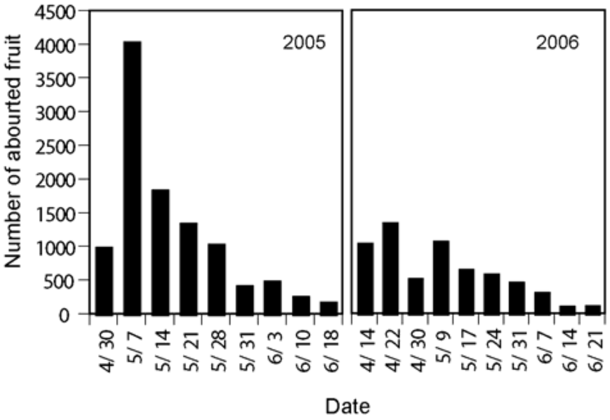


Fig. 3. Patterns of seed abortion throughout the fruiting period in 2005 and 2006.Data from all individuals were

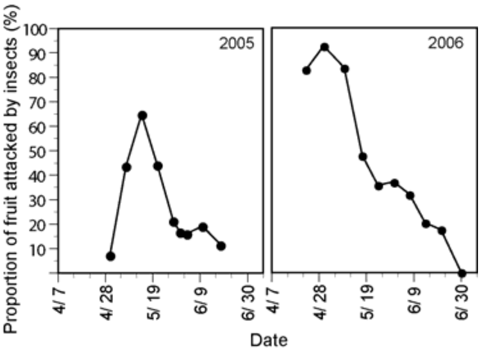


Fig. 4. Proportions of fruit that suffered predation by insects in 2005 and 2006.

Thus, predation by insects occurred only during the early phase of fruit development in 2005. In contrast, in 2006, insects attacked fruit continuously and at a higher intensity than in 2005 from just after flowering onward. From mid-April to mid-May, most of the aborted fruit was infested by insects, with the highest percentage of 92.6% occurring during the week of 23–30 April (Fig. 4). The overall proportion of fruit attacked throughout the fruiting period of 2006 was 50.5%, which was approximately 20% higher than in the previous year. A greater proportion of fruit was attacked in 2006 than in 2005 ($\chi^2 = 175.51$, $p < 0.001$; Table 2).

Table 2. Comparison of the annual percentage of attacked fruits between 2005 and 2006. *** $p<0.001$.

	Number of fruits		χ^2
	Attacked	Non-attacked	
2005	2121	4067	175.51***
2006	540	415	

For the surviving fruit, the proportion of fruit attacked was consistently low throughout the fruiting period, with the highest proportion of 19.6% measured on 8 April. The proportion of attacked fruit differed significantly between surviving and aborted fruit; from early April to early June, the insect predation rate was significantly lower in surviving than in aborted fruit (8–14 April, $\chi^2 = 64.4$, $p < 0.001$; 14–17 May, $\chi^2 = 83.19$, $p < 0.001$; 4–7 June, $\chi^2 = 11.6$, $p < 0.001$; Fig. 5). However, there was no difference after mid-June (15–21 June; $\chi^2 = 0.58$, $p = 0.448$; Fig. 5).

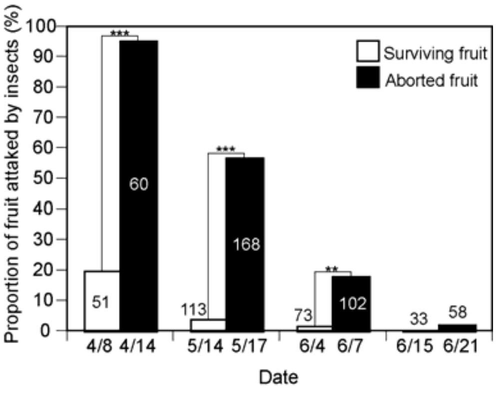


Fig. 5. Comparison of insect attack between living and aborted fruit in 2006. Bars indicate the proportions of aborted attacked (■) and surviving attacked (□) fruit pooled for all six female trees sampled. The numbers in the bars indicate the numbers of dissected fruit. The numbers under the bars indicate the dates on which fruit was collected; living fruit was collected that day and aborted fruit was collected 1 week from that day. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Discussion

Throughout the 2 years of the study, we identified two lepidopteran species that may be major seed predators of *M. rubra*. The emergence of large numbers of *T. pancratiastis* individuals in both years indicates that it is a major seed predator and may play an important role in the pre-dispersal seed predation of *M. rubra* on Yakushima Island. We confirmed that pre-dispersal seed predation by insects occurred mainly during the early phase of fruit development and that predation by *T. pancratiastis* possibly had a significant effect on the survivorship of young fruit. We observed that some individuals of *T. pancratiastis* emerged from one fruit and entered into another one. Therefore, individual larvae may have attacked more than one fruit.

The pattern of fruit survivorship was similar among the three female trees sampled: a dramatic decrease occurred just after flowering, followed by a moderate decrease. Predation by *T. pancratiastis* may partly explain this pattern.

The proportion of living fruit attacked by insects was very low throughout the fruiting period, suggesting that some sound fruit remained on the trees. In contrast, much of the aborted fruit suffered from predation. If fruit abortion occurs at random, there should be no difference in the proportions of living and aborted fruit that are attacked. Mother plants may selectively abort attacked fruit. The Yakushima macaque, which is a major seed disperser of *M. rubra* on Yakushima Island, eats and disperses mature fruit from mid-June to early July. Thus, many non-attacked fruit would be dispersed by Yakushima macaque.

M. rubra exhibits mast fruiting. According to the predator satiation hypothesis, the population density of seed predators becomes small in poor harvest years because of starvation, and in a mast year, plants produce many sound seeds that escape predation because the increase in the predator population density can not catch up with the increase in resource abundance. The crop size of five female *M. rubra* was approximately twice as large in 2005 than in 2006, and seed predation on *M. rubra* was 1.5 times higher in 2006 than in 2005. Thus, mast fruiting may have reduced the predation rate.

However, *T. pancratiastis* attacks not only fruit, but also new leaves. *T. pancratiastis* stays in the leaf veins and eats the new leaf tissue. Before pupating, it severs the new leaf in which it occurs and falls to the ground with the leaf tip. On the ground, it makes a nest out of the leaf tip and pupates inside the nest (personal observations). From early June to late June, we collected these nests from the seed traps and reared 283 adult *T. pancratiastis* from 554 pupae. Thus, *T. pancratiastis* may maintain its population density by using new leaves in years of limited fruiting. To evaluate the effect of pre-dispersal seed predation on *M. rubra*, it is important to understand the life history of seed predators and their resource use.

References

Agetsuma N (1995) Dietary selection by Yakushima macaques (*Macaca fuscata yakui*): the influence of food availability and temperature. International Journal of Primatology 16: 611–627.

Ida H, Hotta M, Ezaki Y (2004) Predispersal seed predation by rodents to beechnuts (*Fagus crenata* Blume). Ecological Research 19: 503–509.

Janzen DH (1971) Seed predation by animals. Annual Review of Ecology and Systematics 2: 465–492.

Nakagawa M, Takeuchi Y, Kenta T, Nakashizuka T (2005) Predispersal seed predation by insects vs. vertebrates in six dipterocarp species in Sarawak, Malaysia. Biotropica 37(3): 389–396.

Sun S, Gao X, Chen L (2004) High acorn predation prevents regeneration of *Quercus liaotungensis* in the Dongling Mountain region of North China. Restoration Ecology 12 (3): 335–342.

Tagawa H (1980) Vegetation on the western slope of Mt. Kuniwaridake, Yakushima Island. Science Reports of Kagoshima University 29: 121–137. (in Japanese)

Effects of Forest Fragmentation on Tree Regeneration from Bird-Dispersed Seeds in a Temperate Forest in Japan

Shoji Naoe[1], Shoko Sakai[1], Ayako Sawa[2], Takashi Masaki[3]
[1] *Center for Ecological Research, Kyoto University, Hirano 509-3, Otsu, 520-2113, Japan*
[2] *Graduate School of Life and Environmental Sciences,*
University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan
[3] *Forestry and Forest Prodcuts Research Institute, Matsunosato 1,*
Tsukuba, Ibaraki 305-8687, Japan

Introduction

The loss and fragmentation of natural habitats caused by human activities are pervasive phenomena in terrestrial ecosystems and are considered to be major threats to biodiversity (Fisher & Lindenmayer 2007). Today, habitat fragmentation has become one of the most important research themes in conservation biology. With increased levels of research, our understanding of the processes involved in fragmentation and the effects of fragmentation on habitats has developed considerably over recent decades (Hobbs & Yates 2003). Important research advances include results from long-term fragmentation experiments (e.g., Bierregaard et al. 2001), the elucidation of the variety of effects caused by the creation of edges between fragments and surrounding altered land (e.g., Laurence 2000), and detailed considerations of the genetic and demographic consequences of fragmentation (e.g., Young & Clarke 2000; Isagi et al. 2007) and the alteration of plant–animal interactions, especially in plant reproductive processes (e.g., Aguilar et al. 2006).

Despite these advances, we are still a long way from developing a comprehensive conceptual framework for how forest fragmentation influences community composition, species diversity, and the dynamics of individual species (Hobbs & Yates 2003) for several reasons. First, the effects of fragmentation strongly depend on the characteristics of the focal ecosystem and the type of fragmentation (area, surrounding conditions, etc.). We cannot apply results for a tropical forest to a temperate forest, or those from a primary forest to a secondary forest. Fragmentation caused by land-use changes from forest to tree plantation may be entirely different from that caused by the expansion of agricultural lands. Second, many observations and experiments report changes after fragmentation or differences between continuous and fragmented forests, but it is difficult to provide clear insights into the ecological mechanisms of the changes or differences. Third, many studies examine only one or a few aspects of effects of the fragmentation. For the consideration of the long-term and total effects of forest fragmentation on biodiversity and ecosystem functions, pervasive studies of all biological processes of a species or forest are essential. In the case of plants, studies of the effects on reproductive processes such as pollination are abundant, whereas few studies have examined the effects on seedling survival and growth (Hobbs & Yates 2003).

Therefore, we investigated the effects of habitat fragmentation in a community of trees that use birds for seed dispersal in temperate forests in Japan. Although most dominant tree species in temperate forests are wind-pollinated and wind-dispersed, bird-dispersed tree species (which are mainly pollinated by insects) are important because they provide food for birds and mammals, play important roles in forest regeneration and forest succession, and maintain high species diversity. We monitored several biological processes using various methods in these forests: species composition in permanent plots; fruiting and dispersal using seed traps; the activities of seed dispersers by observing birds; and seedling germination and demography in permanent plots. Here, we report preliminary results.

Methods

Study site

The study was conducted in a mixed deciduous forest, the Ogawa Forest Reserve, at the southern edge of the Abukuma mountain region, central Japan (36º56' N, 140º35' E; 600–660 m above sea level). The reserve consists of a mixed deciduous old-growth forest of 98 ha (conserved forest) and remaining strips of old-growth forest approximately 50 m wide (fragments), surrounded by evergreen conifer plantations, secondary forests, and agricultural lands (pastures and vegetable or paddy fields; Fig. 1). The annual precipitation is approximately 1750 mm, and the mean annual temperature is 9.0°C, with an average monthly temperature range of –1.6°C in February to 20.5°C in August.

In the conserved forest, the total basal area and density of trees > 5 cm in diameter at breast height (DBH) were 33 m^2 ha^{-1} and 850 stems ha^{-1}, respectively (Masaki et al. 1992). The dominant tree species in terms of total basal area were *Quercus serrata* (27%), *Fagus japonica* (20%), and *F. crenata* (9%). Dwarf bamboos (*Sasa*, *Sasaela*, and *Sasamorpha* spp.) covered parts of the forest floor. Disturbances related to human activity, grazing, and fire, affected the forest until the 1930s, especially at the margins of the forest reserve (Suzuki 2002). The fragments are similar in composition and structure to the old-growth forest; however, they are small in area because large parts of the old-growth forest were cut during the 1970s and were converted into conifer plantations. The plantations are pure stands of *Cryptomeria japonica* or *Chamaecyparis obtusa*. The remaining area is covered by secondary forests and agricultural fields. The secondary forests have been managed for the production of firewood and charcoal for several decades. The dominant secondary forest species are *Q. serrata*, *Pinus densiflora*, and *Carpinus turczaninovii*.

Monitoring of plants

A 6-ha permanent plot (200 x 300 m) was established in 1987 in the central part of the Ogawa Forest Reserve (Fig. 1). The plot has been censused regularly for factors such as light, topography, and tree demography and growth within the plot (Tanaka & Nakashizuka 2002). In addition, we established two 1-ha plots in the fragmented forest area in 2006 (Fig. 1). All trees with DBH > 5 cm were measured, identified, and tagged. The plots in the conserved forest and the fragment have 1 x 1 m quadrats located at every 10 x 10 m grid point (total: 651 quadrats in the conserved forest and 132 in the fragment). In July 2006 and July 2007, we marked and monitored all current-year seedlings of bird-dispersed species in the quadrats. Seedling survival in 2006 was censused in October. To monitor seed rain and seed dispersal, we placed 329 and 67 seed traps at regular spacing in the plots of the conserved forest and the fragment, respectively. Seeds of bird-dispersed species were collected from the seed traps twice a month from July to December 2006 and identified to species. Seeds that were still covered with fleshy parts such as the mesocarp or aril probably dropped without being dispersed by birds; therefore, seeds that had lost the fleshy parts after being eaten and excreted by birds were distinguished and counted separately.

Monitoring of birds

To assess numbers and composition of bird dispersers, we conducted bird censuses within the plots and in an additional census site in the fragment from July to December 2006. We counted and identified birds passing through a 40 x 100 m area within a 15-min period. Each census was conducted early in the morning (first 3 h after dawn) and repeated three times at different points in the conserved forest and the fragment to obtain data for each census, except for three of the censuses in the fragment, which were only conducted twice. The censuses were done two to ten times a month, and more censuses were done in the bird migration season. In total, 33 censuses were completed in 2006. Frugivorous birds, which were the potential dispersers, were identified based on the literature (e.g., Kiyosu 1966; Kanouchi 2006). Differences in the densities of bird dispersers between the conserved forest and the fragment were examined using analysis of variance (ANOVA), with month and site as dependent variables.

Results

We compared the basal area of bird-dispersed woody species between the conserved forest and the forest fragment. For the bird-dispersed species *Prunus grayana, Ilex macropoda,* and *Eleutherococcus sciadphylloides*, the basal area in the fragment was more than twice that in the conserved forest (Table 1). The difference was especially large in *E. sciadphylloides*, at 9.3 times.

We compared the relative seed production per unit tree basal area for the three bird-dispersed species *Prunus verecunda, I. macropoda,* and *E. sciadphylloides,* for which > 30 seeds were trapped (Table 2). In all three species, the relative seed production was greater in the fragment than in the conserved forest, and the differences were significant. In contrast, the proportion of seeds dispersed by birds tended to be lower in the fragment than in the conserved forest, except for the liana *Rhus ambigua* (Table 3). The difference was significant for two tree species: *I. macropoda* and *E. sciadphylloides*.

We found 10 or more seedlings of at least 1 year old for six species in the two plots. The seedling densities of *E. sciadphylloides* and *Rhus ambigua* were considerably higher in the fragment than in the conserved forest in both study years. There was a significant difference in the survival of current-year seedlings only for *Cornus controversa*, for which the numbers were higher in the fragment than in the conserved forest (Table 4).

The number of bird species, frugivorous species, and bird diversity measured by the Shannon diversity index (H') did not differ significantly between the conserved forest and the fragment (Table 5). The number of frugivorous individuals was slightly greater in the fragment than in the conserved forest.

Discussion

Although these preliminary analyses were mostly based on data obtained in a single year, and 2006 was a poor year in terms of seed production (T. Masaki, unpublished data), we did detect some potential effects of fragmentation on seed production, seed dispersal, and seedling survival. Considering the relatively large differences in the basal area of some of the tree species, these effects may have already caused differences in the species composition of mature trees in the 30 years since forest fragmentation. Laurence et al. (2006) similarly reported a significant increase in trees with a DBH of 10–20 cm along forest edges 22 years after fragmentation. These effects may be positive or negative, depending on the processes and species involved. Positive effects of forest fragmentation were observed in seed production per unit tree basal area (Table 2) and in seedling survival for some species (Table 4). In terms of plant species reproduction, Aguilar et al. (2006) found an overall large negative effect of forest fragmentation on pollination and plant reproduction that was probably caused by pollination limitation. Positive effects have rarely been reported (e.g., Aizen & Feinsinger 1994). Our contradictory results may have occurred partly because our fragment has relatively large forests in close proximity, and the pollinator fauna may thus be little affected. In addition, the edge effect may have improved light conditions within the fragment, resulting in greater resources available for reproduction. The location of the fragment, i.e., on ridges or close to roads and rivers, could also be responsible for the better light conditions.

Unlike pollination, few studies have investigated the effect of fragmentation on seed dispersal and seedling density. Farwig et al. (2006) reported a marginally lower density of frugivorous birds, but significantly higher seed removal in forest fragments than in continuous forests, probably because of the paucity of other available fruit resources. Some studies have measured declines in the density of birds or frugivores with fragmentation (e.g., Andrén 1994; Cordeiro & Howe 2003; Luck & Daily 2003). Others have reported that forest fragmentation results in edge effects, namely high rates of nest predation and parasitism near forest edges, that can threaten bird populations by reducing nesting success in the remnant forest habitats (Batáry & Báldi 2004; Hoover et al. 2006). Our bird censuses indicate that bird densities or activities were slightly higher in the forest fragment. However, considering the difference in the amount of fruit, which was higher in the fragment, the difference is relatively small and may explain the lower proportion of dispersed seeds in the fragments than in the conserved forest. One further important consideration is that it may be more useful to examine actual dispersal patterns, rather than simply comparing the proportion of dispersed seeds (Schupp 1993). Further analyses, by combining examinations of seed dispersal, germination, and seedling survival over more than 1 year, will be important.

Our preliminary results reveal the importance of edge effects on the regeneration of bird-dispersed tree species such as an increase in fruit resources associated with an increase in frugivorous birds leading to an improvement in the survivorship of seedlings. It is essential to have a complete life history of the area and its species to understand the total effects and long-term results of forest fragmentation. The susceptibility to the effects of fragmentation may vary among tree species. Contrary to tropical forests, which are dominated by animal-dispersed trees, many bird-dispersed tree species that occur in temperate forests are mid-successional species. These species may be more robust than tropical species in their responses to forest fragmentation.

References

Aguilar R, Ashworth L, Galetto L, Aizen MA. 2006. Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. Ecol Letters 9:968-980

Aizen NA, Feinsinger P. 1994. Forest fragmentation, pollination, and plant reproduction in a Chaco dry forest, Argentina. Ecology 75:330-351

Andrén H. 1994. Effects of habitat fragmentation on birds and mammals in landscapes with different proportions of suitable habitat – a review. Oikos 80:193-196

Batáry P, Báldi A. 2004. Evidence of an edge effect on avian nest success. Conserv Biol 18:389-400

Bierregaard RO, Gascon C, Lovejoy TE, Mesquita R (eds). 2001. Lessons from Amazonia: the ecology and conservation of a fragmented forest. Yale University Press, New Haven, CT

Cordeiro NJ, Howe HF. 2003. Forest fragmentation severs mutualism between seed dispersers and an endemic African tree. Proc Nat Acad Sci 100:14052-14056

Farwig N, Böhning-Gaese K, Bleher B. 2006. Enhanced seed dispersal of *Prunus africana* in fragmented and disturbed forests. Oecologia 147:238-252

Fisher J, Lindenmayer DB. 2007. Landscape modification and habitat fragmentation: a synthesis. Global Ecol Biogeog 16:265-280

Hobbs RJ, Yates CJ. 2003. Impacts of ecosystem fragmentation on plant populations: generalising the idiosyncratic. Aust J Bot 51:471-488

Hoover JP, Tear TH, Baltz ME. 2006. Edge effects reduce the nesting success of Acadian Flycatchers in a moderately fragmented forest. J Field Ornithology 77:425-436

Isagi Y, Tateno R, Matsuki Y, Hirao A, Watanabe S, Shibata M. 2007. Genetic and reproductive consequences of forest fragmentation for populations of *Magnolia obovata*. Ecol Res 22:382-389

Kanouchi T. 2006. The handbook of birds and fruits. (in Japanese). Bunichi-sougou-shuppan, Tokyo

Kiyosu Y. 1966. Encyclopedia of wild birds. (in Japanese). Tokyodo-shuppan, Tokyo

Laurence WF. 2000. Do edge effects occur over large spatial scales? Trends Ecol Evol 15:134-135

Laurence WF, Nascimento HEM, Laurance SG, Andrade A, Ribeiro JELS, Giraldo JP, Lovejoy TE, Condit R, Chave J, Harms KE, D'Angelo S. 2006. Rapid decay of tree-community composition in Amazonian forest fragments. Proc Nat Acad Sci 103: 19010-19014

Luck GW, Daily GC. 2003. Tropical countryside bird assemblages: richness, composition, and foraging differ by landscape context. Ecol Appl 13:235-247

Masaki T, Suzuki W, Niiyama K, Iida S, Tanaka H, Nakashhizuka T. 1992. Community structure of a species-rich temperate forest, Ogawa Forest Reserve, central Japan. Vegetatio 98:97-111

Schupp EW. 1993. Quantity, quality and the effectiveness of seed dispersal by animals. Vegetatio 107:15-29

Suzuki W. 2002. Forest vegetation in and around Ogawa Forest Reserve in relation to human impact. In: Nakashizuka T, Matsumoto Y (eds), Diversity and interaction in a temperate forest community. Ecological Studies 158, 27-40. Springer-Verlag, Tokyo

Tanaka H, Nakashizuka T. 2002. Ground design of the research site. In: Nakashizuka T, Matsumoto Y (eds), Diversity and interaction in a temperate forest community. Ecological Studies 158, 43-49. Springer-Verlag, Tokyo

Young AG, Clarke GM (eds). 2000. Genetics, demography and viability of fragmented populations. Cambridge University Press, Cambridge, UK

TABLE 1. Basal area of major tree species in plots in the conserved forest (6-ha plot) and forest fragment (two 1-ha plots).

	Basal area (cm^2/ha)	
Species	Conserved forest	Fragment
Bird-dispersed plants		
Prunus verecunda	9249	4266
Prunus grayana	508	1632
Ilex macropoda	1020	2419
Cornus controversa	13411	9737
Eleutherococcus sciadphylloides	805	7512
Kalopanax septemlobus	7989	4027
Other plants		
Fagus crenata	29102	20904
Fagus japonica	63330	11615
Quercus serrata	93457	76164
Quercus crispula	11005	51239
Castanea crenata	15022	63890
Styrax obassia	8653	5421
Acer amoenum	12209	14411
Acer mono	11350	3708
Carpinus laxiflora	15108	19493

TABLE 2. Relative seed production per unit basal area in the conserved forest and the forest fragment in 2006. Tree species with 30 > seeds for both forests are included.

	Relative seed production (no./cm^2)	
Species	Conserved forest	Fragment
Prunus verecunda	0.016	0.020**
Ilex macropoda	0.067	0.094**
Eleutherococcus sciadphylloides	0.011	0.020**

***p* < 0.01, chi-square test for independence.

TABLE 3. Comparison of seed removal rates between the conserved forest and forest fragment in 2006. Species with 30 > seeds for both forests are included.

Species	Conserved forest	Fragment
Prunus verecunda	0.17	0.15
Ilex macropoda	0.59**	0.44
Eleutherococcus sciadphylloides	0.53**	0.25
Rhus ambigua	0.52	0.66

***p* < 0.01, chi-square test for independence.

TABLE 4. Density of current-year seedlings in July 2006 and 2007 in the conserved forest and forest fragment, and survival rates of the seedlings from July to October 2006.
Species with 10 > seedlings in either year are included.

	Seedling emergence (no./m^2)				Survival rate (Jul-Oct 2006)	
	2006		2007			
Species	Conserved forest	Fragment	Conserved forest	Fragment	Conserved forest	Fragment
Ilex macropoda	-	-	0.03	0.08	-	-
Cornus controversa	0.31	0.40	0.85	0.65	0.08	0.25**
E. sciadphylloides	0.07	1.09	0.07	0.89	0.57	0.41
Kalopanax septemlobus	-	-	0.57	0.19	-	-
Rhus ambigua	0.04	0.08	0.36	1.11	0.19	0.10
Euonymus oxiphyllus	0.26	0.13	0.40	0.05	0.4	0.29

**$p < 0.01$, chi-square test for independence.

TABLE 5. Comparison of the number and diversity of birds between the conserved forest and the forest fragment observed from July to December 2006.

	Conserved forest	Fragment
Number of bird species	26	27
Shannon diversity index (H')	2.64	2.58
Number of frugivorous species	22	22
Number of frugivorous individuals	283	344*

*$p < 0.05$, one-way ANOVA.

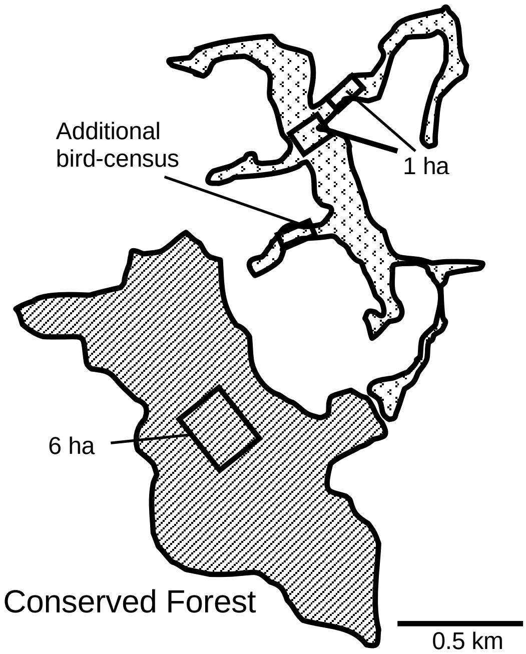


FIGURE 1. Location of plots and bird census sites in the conserved forest and the forest fragment in Ogawa Forest Reserve.

Effects of Deforestation on Mutualistic Interactions of Ants with Plants and Hemipterans in a Tropical Rain Forest of Borneo

Hiroshi O. Tanaka[1], Seiki Yamane[2], Kuniyasu Momose[3], Takao Itioka[1]
[1] *Graduate School of Human and Environmental Studies*
Kyoto University, Kyoto 606-8501, Japan
[2] *Faculty of Science, Kagoshima University, Kagoshima 890-0065, Japan*
[3] *Faculty of Agriculture, Ehime University, Matsuyama 790-8566, Japan*

Introduction

In Southeast Asia, the area of lowland tropical rain forests has decreased rapidly as a result of deforestation by humans, resulting in the creation of several types of secondary forest (Sodhi et al. 2004). This decrease has been accompanied by a dramatic loss of species richness. So far, deforestation-related effects of humans on tropical rain forest biodiversity have been evaluated only by determining the species losses caused by deforestation (e.g., Levings and Windsor 1985; Lawton et al. 1998; Dunn 2004). To better understand the effects of deforestation on biodiversity, however, we must explore not only the effects on species richness, but also those on interspecific interactions within a biological community; interspecific interactions are likely to be involved in the processes and mechanisms that allow species to coexist in the community. Therefore, it is important to clarify differences in the properties of interspecific interactions, as well as differences in species richness, in relation to the degree of deforestation.

Ants have often been used as indicators of the effects of forest disturbance on biodiversity (e.g., Belshaw and Bolton 1993; Brühl et al. 2003; Bickel et al. 2006) because they dominate the terrestrial and arboreal arthropod fauna of tropical forests (Stork 1988; Belshaw and Bolton 1993; Floren and Linsenmair 1997) and are considered to contribute to local species richness through their involvement in various types of interactions, including mutualistic interactions with a wide taxonomic range of organisms. Ant plants (myrmecophytes), plants with extrafloral nectaries (EFNs), and honeydew-producing hemipterans commonly enter into mutualistic relationships with ants in the tropics (Buckley 1982; Hölldobler and Wilson 1990; Oliveira and Oliveira-Filho 1991; Koptur 1992; Davidson and McKey 1993; Davidson 1998).

What effect does deforestation have on such mutualistic interactions? To date, few studies have addressed this question by focusing on changes in the properties of ant-associated mutualistic interactions caused by deforestation (e.g., Bruna et al. 2005), although some studies have attempted to estimate the effects of deforestation on biodiversity by measuring the loss of ant species (e.g., Belshaw and Bolton 1993; Brühl et al. 2003). The main purpose of the present study was thus to describe how the properties of ant-associated mutualistic interactions differ among forests of different stand ages (i.e., different elapsed time since the last slash-and-burn event) in a given locality. For this purpose, we measured the frequency of occurrence and the species composition involved in mutualistic interactions between ants and plants or hemipterans in primary and secondary forest plots differing in stand age. By comparing these parameters among forest plots, we attempted to examine the effects of deforestation on the interactions of ants with plants and hemipterans in tropical rain forests.

Methods

The study was conducted from August to September 2003 in a primary forest of the Lambir Hills National Park, Sarawak, Malaysia and in secondary forest of differing stand age around the outside of the park. We chose 15 secondary forest plots of three stand types for our survey: four plots of 1-year-old secondary forest stands (abbreviated as 1SF), five of 5-year-old secondary forest (5SF), and six of old secondary forest (>19 years old; OSF). We established one 100×10 m study plot at or near the center of each of the 15 secondary forest stands. In the park's primary mixed dipterocarp forest, we established four shaded primary forest study plots (SPF) in the same way as in the secondary forest stands. Most of the forest floor inside the four plots was relatively shady, with a thick canopy layer, and no obvious forest gaps were included in the plots. We also selected two gaps approximately 10 m in diameter near the four shaded plots. Then, the 2 m-wide fringes of the gaps were set in the additional study plots (GPF) for gaps in the primary forest.

We conducted a field census of ants found attending EFNs, hemipterans, and myrmecophytes in each study plot. Except for the GPF plots, each 100×10 m plot was divided into 40 subplots of 25 m^2 each. In each subplot, we randomly selected 10 tree saplings or young trees that were 0.5 to 2.0 m in height. In each GPF plot, we randomly selected 400 tree saplings or shrubs 0.5 to 2.0 m in height. In several plots, the total number of census trees ranged between 400 and 410 because of miscounts.

During each census, we checked whether ants were present attending EFN glands or hemipterans on the selected trees. Lianas were omitted from the census because it was difficult to identify them. EFNs and hemipterans that were not attended by ants were omitted from our census. We also checked whether the selected trees were myrmecophytes by searching for domatium-like structures that ants could inhabit and for the presence of any entrance and exit holes for ants; in addition, we tested whether ant symbionts had been recruited by manually shaking the trees. We also checked whether the weaver ant *Oecophylla smaragdina* (Fabricius) (Formicinae) was present on the selected trees because this species maintains large territories around arboreal nests located on multiple trees in which it excludes other ants from any EFNs and hemipterans present in the territory (Hölldobler and Wilson 1990; Blüthgen and Fiedler 2002).

We calculated the frequency of each association as the percentage of all sampled trees (ca. 400) in a plot on which a given association was recorded. We compared the frequencies of EFN-bearing trees on which the EFNs were attended by ants, of trees with hemipteran-attending ants, of myrmecophytes, and of trees with *O. smaragdina*, among the five types of forest stand (1SF, 5SF, OSF, SPF, and GPF) using a *G*-test. We also calculated the average numbers of species of EFN-attending ants, hemipteran-attending ants, trees that harbored such interactions, and myrmecophytes in each forest type.

In addition to comparisons among forest stands of different ages, we assessed the inferred effect of deforestation on ant-associated interactions by calculating the above-mentioned variables for primary and secondary forest vegetation as separate categories. To estimate the properties of ant-associated interactions for primary forest as a whole (PF), we combined the data from the GPF and SPF forest types. To estimate the properties for the secondary forest as a whole (SF), we combined the data from the three secondary-forest types (1SF, 5SF, and OSF).

Results

The frequency of trees on which EFNs were attended by ants differed significantly among forest stand types (*G* = 180.05, *P* < 0.001). The mean frequencies of EFN-bearing trees attended by ants (3.2 to 5.6%, respectively) were conspicuously higher in GPF and 1SF than in 5SF, OSF, and SPF (<0.5% in each). However, the mean frequency of EFN-bearing trees attended by ants did not differ between PF and SF (*G* = 1.02, *P* = 0.30).

The average number of ant species per plot found attending EFNs in PF (3.7± 2.0 species per plot) was higher than that in SF (1.3 ± 0.7). The average number of ant species was highest in GPF (10.0 ± 1.0) and second-highest in 1SF (4.3 ± 2.1). For 5SF, OSF, and SPF, an average of only 0.2 to 0.5 species of ants were observed attending EFNs. No ant species attended EFNs in both PF and SF.

The average number of species of EFN-bearing trees per plot in PF (2.7 ± 2.0) was higher than that in SF (0.4 ± 0.2). The average number of species of EFN-bearing trees was highest in GPF (7.0). In the other forest stand types, only 0.2 to 1.0 species of EFN-bearing trees per plot were found. Of all the species of EFN-bearing trees that were observed in PF, only 13% were also observed in SF. In 1SF, 96% of all 52 EFN-bearing trees that we observed belonged to one species (*Homalanthus populneus* (Geiseler), Euphorbiaceae), and most trees were attended by one of two ant species: *Crematogaster* sp. 85 (Myrmicinae) and *Tapinoma* sp. 1 (Dolichoderinae).

The frequency of trees on which hemipterans were attended by ants differed significantly among forest stand types (*G* = 48.01, *P* < 0.001). The mean frequencies in 5SF, GPF, and SPF ranged from 1.5 to 2.6%, whereas those in OSF and 1SF were <0.65%. The mean frequency did not differ between PF and SF (*G* = 1.59, *P* = 0.20).

The average number of ant species per plot attending hemipterans in PF (3.5 ± 0.7) was higher than that in SF (0.9 ± 0.3). The average number of ant species was highest in GPF (4.0 ± 1.0) and second-highest in SPF (3.3 ± 1.0). In 1SF, 5SF, and OSF, an average of 0.8 to 1.2 species of ants were observed attending hemipterans. Of all the species of ant that were observed in PF, only 19% were also observed in SF.

The average number of tree species per plot on which hemipterans were attended by ants in PF (2.8 ± 0.5) was higher than that in SF (0.6 ± 0.2). The average number of tree species was highest in GPF (3.0 ± 1.0) and second-highest in SPF (2.8 ± 0.8). In each of the three types of secondary forest stand, fewer tree species were found per plot than in any type of primary forest stand. No tree species on which hemipterans were attended by ants was observed in both PF and SF. In 5SF, *Melastoma malabathricum* (Melastomataceae) accounted for about 89% of the 53 trees with hemipterans that were attended by ants of one species, *Dolichoderus affinis* (Dolichoderinae), which was also observed on other trees.

All 10 species of myrmecophytic trees belonged to the genus *Macaranga* (Euphorbiaceae). The frequency of myrmecophytes differed significantly among forest stand types (*G* = 248.55, *P* < 0.001). The frequency was higher in GPF than in any other forest stand type, and was about seven times the value in 5SF, which had the second-highest frequency. The frequencies in OSF, 1SF, and SPF were less than 7%, 4%, and 2% of the frequency in GPF, respectively. The total frequency of *Macaranga* myrmecophytes in PF was significantly higher (6.2 versus 1.5%) than that in SF (*G* = 23.80, *P* < 0.001).

The average number of myrmecophytic *Macaranga* species per plot in PF (3.2 ± 1.6) was higher than that in SF (1.1 ± 0.3). The average number of species of myrmecophytic *Macaranga* was highest in GPF (8.0). In the other forest stand types, only 0.8 to 1.6 species of myrmecophytic *Macaranga* per plot were found. All *Macaranga* myrmecophytes observed in SF were also found in PF. In 5SF, *Macaranga bancana* accounted for 89% of the 56 *Macaranga* individuals. The occurrence of this species was higher in SF (82 individuals) than in PF (24 individuals).

The frequency of trees occupied by *O. smaragdina* differed significantly among forest stand types (G = 126.06, $P < 0.001$). The frequency was notably higher in 1SF (3.5% ± 2.1%) than in the other forest stand types (0.7% ± 0.6% for 5SF, 0.3% ± 0.3% for OSF, 0% for SPF, and <0.01% for GPF) and tended to decrease as stand age increased. The total frequency of trees occupied by *O. smaragdina* in SF was significantly higher than that in PF (1.3 vs. <0.01%; G = 36.97, $P < 0.001$).

Discussion

Our results demonstrate that slash-and-burn deforestation affects the properties of ant-associated mutualistic interactions. For ants attending both EFNs and hemipterans, for trees bearing EFNs, and for trees with hemipteran-attending ants, the number of species was higher in primary than in secondary forest, and fewer than 20% of the species observed in the primary forest plots were also recorded in the secondary forest plots. For *Macaranga* myrmecophytes, both the number of species and the frequency of occurrence were higher in primary than in secondary forest, and the species observed in secondary forest comprised approximately one-third of the species occurring in primary forest. In contrast, the weaver ant *O. smaragdina*, which tended to exclude other arboreal ant species, was significantly more abundant in secondary than in primary forest. These results suggest that slash-and-burn deforestation drastically decreases the diversity of species involved in ant-associated mutualistic interactions and simplifies the composition of the involved species. We also found that these effects of deforestation lasted for 19 years.

Acknowledgements

We thank the Forest Research Center and Forest Department of Sarawak, Malaysia for their approval for our research plan. We also thank Dr. Katsuyuki Eguchi for identification of Pheidole, Ms. Lucy Chong, Mr. Het Kaliang and Mr. Paulus Meleng for support on our study. This study was supported by a Project of the Research Institute for Humanity and Nature (P2-2)

References

Belshaw R, Bolton B (1993) The effect of forest disturbance on the leaf litter ant fauna in Ghana. Biodiversity Conserv 2(6):656-666

Bickel TO, Brühl CA, Gadau JR, Hölldobler B, Linsenmair KE (2006) Influence of habitat fragmentation on the genetic variability in leaf litter ant populations in tropical rainforests of Sabah, Borneo. Biodiversity Conserv 15(1):157-175

Blüthgen N, Fiedler K (2002) Interactions between weaver ants *Oecophylla smaragdina*, homopterans, trees and lianas in an Australian rain forest canopy. J Anim Ecol 71(5):793-801

Brühl CA, Eltz T, Linsenmair KE (2003) Size does matter – effects of tropical rainforest fragmentation on the leaf litter ant community in Sabah, Malaysia. Biodiversity Conserv 12(7):1371-1389

Bruna EM, Vasconcelos HL, Heredia S (2005) The effect of habitat fragmentation on communities of mutualists: Amazonian ants and their host plants. Biol Conserv 124(2):209-216

Buckley RC (1982) Ant-plant Interactions in Australia. Dr. W. Junk Publishers, The Hague

Davidson DW (1998) Resource discovery versus resource domination in ants: a functional mechanism for breaking the trade-off. Ecol Ent 23(4):484-490

Davidson DW, McKey D (1993) The evolutionary ecology of symbiotic ant-plant relationships. J Hymenop Res 2(1):13-83

Dunn RR (2004) Recovery of faunal communities during tropical forest regeneration. Conserv Biol 18(2):302-309

Floren A, Linsenmair KE (1997) Diversity and recolonisation dynamics of selected arthropod groups on different tree species in a lowland rain forest in Sabah, Malaysia. In: Stork NE, Adis J, Didham RK (eds). Canopy arthropods. Chapman & Hall, London, pp 344-381

Hölldobler B, Wilson EO (1990) The ants. The Belknap Press of Harvard University Press, Cambridge, Massachusetts

Koptur S (1992) Extrafloral nectary-mediated interactions between insects and plants. In: Bernays E (ed). Insect-plant interactions. CRC Press, Florida, pp 81-129

Lawton JH, Bignell DE, Bolton B, Bloemers GF, Eggleton P, Hammond PM, Hodda M, Holt RD, Larsen TB, Mawdsley NA, Stork NE, Srivastava DS, Watt AD (1998) Biodiversity inventories, indicator taxa and effects of habitat modification in tropical forest. Nature 391(6662):72-76

Levings SC, Windsor DM (1985) Litter arthropod populations in a tropical deciduous forest: relationships between years and arthropod groups. J Anim Ecol 54(1):61-69

Oliveira PS, Oliveira-Filho AT (1991) Distribution of extrafloral nectaries in the woody flora of tropical communities in Western Brazil. In: Price PW, Lewinsohn TM, Fernandes WG, Benson WW (eds). Plant-animal interactions. John Wiley & Sons, New York, pp 163-175

Sodhi NS, Koh LP, Brook BW, Ng PKL (2004) Southeast Asian biodiversity: an impending disaster. Trends Ecol Evol 19(12):654-660

Stork NE (1988) Insect diversity: facts, fiction, and speculation. Biol J. Linn Soc 35(4):321-338

Effects of Canopy Gaps on Ant–Hemiptera–Plant Interactions in Lambir Hills National Park, Sarawak

Chihiro Handa[1], Takao Itioka[1], Seiki Yamane[2], Hidetoshi Nagamasu[3]
[1]*Graduate School of Human and Environmental Studies, Kyoto University*
[2]*Department of Science, Kagoshima University*
[3]*The Kyoto University Museum, Kyoto University*

Introduction

When a tree dies in a closed-canopy forest, it creates a "canopy gap", which is the initial stage of a mini-successional sequence called gap-phase regeneration, culminating in the replacement of the original canopy tree by one or more new trees (Hubbell et al. 1999). Over the past few decades, a considerable number of studies have been done on tree species in canopy gaps (Lawton and Putz 1988; Denslow 1995; Hubbell et al. 1999). Canopy gaps are considered to provide seedlings of many tree species with micro-environmental conditions suitable for their growth (Denslow 1995). Since light is relatively intense in canopy gaps compared with that in the area surrounding gaps, the growth performance of seedlings of most tree species, including pioneer trees that can hardly survive and grow under the forest canopy, is high. Canopy gaps give r-type individuals, which invest little in survivorship or consume resources heavily, a chance to reproduce. Hence gap formation is considered to contribute to the maintenance of tree species diversity and substantially affect the ecological and evolutionary dynamics of many tropical forests (Denslow 1995).

Taking into account that secondary production depends on primary production (Brylinski and Mann 1973; Cole et al. 1988), gap formation is expected to influence organisms that interact with trees. Because areas under canopy gaps have continuously higher resource availability as compared with those under a closed canopy, Levey (1990) categorized canopy gaps as "keystone habitats" for frugivores. However, little is understood about the effects of canopy gaps on the diversity and dynamics of a community of organisms other than the trees. A few studies have evaluated the effects of canopy gaps on fauna or interactions between trees and other organisms, including work on plant–frugivore interactions (Levey 1988, 1990; Beck et al. 2004) and on plant–herbivore interactions (Coley 1983). Thus, the effects of canopy gaps and their role as "keystone habitats" for organisms other than trees have not been adequately studied.

Ants (Hymenoptera: Formicidae) comprise up to 94% of arthropods in insecticidal fogging samples from tropical rain forest canopies and 86% of the biomass of those samples (Tobin 1995; Davidson 1997). Ants often interact with plants both directly over extrafloral nectaries (EFNs) and indirectly over honeydew excreted by myrmecophilous insects such as hemipterans or lepidopteran caterpillars. Ants attend such myrmecophilous insects or EFNs to collect honeydew or nectar, and they protect the myrmecophilous insects or EFN-bearing plants from natural enemies (Hölldobler and Wilson 1990). Thus, ant–hemipteran and ant–plant interactions are sometimes known as mutualisms. Since ants or hemipterans have a close relationship to plants, they would be affected by canopy gaps possessing high plant productivity. However, there is little understanding of the effects of canopy gaps upon ants through their strong relationship with plants or hemipterans. In this study, we were concerned with the effect of canopy gaps on ant–hemipteran–plant interactions.

Materials and Methods

Study site

The data were collected around the Canopy Biology Plot (8 ha) and the Crane Plot (4 ha) in Lambir Hills National Park, Sarawak, Malaysia (4°2'N, 113°50'E; 50–200 m a.s.l.). The park is located about 10 km inland from the coast in the northern part of Sarawak, and the climate is humid tropical, with a constant high temperature and a weak seasonal change in rainfall (Kato et al. 1995). Most of the park is covered with primary evergreen forests, much of which is classified as lowland mixed dipterocarp forest, thought to be one of the richest forests in the world in terms of the diversity of tree species. The canopy of this multi-layered forest, which has developed on nutrient-poor sandy or clayey soil, is about 35 to 40 m high, with emergent trees penetrating the canopy layer attaining heights of more than 70 m (Kato et al. 1995).

In Lambir Hills National Park, 119 families, 437 genera, and 1153 species of vascular plants were identified by Nagamasu and Momose (1997), and more than 257 ant species have been recorded by Yamane and Nona (1994). Yamane and Nona (1994) also showed that the ant fauna on the ground is different from that on tree trunks (ca. 1 to 1.5 m above the ground).

Ant–hemiptera–plant interactions

There were some open canopy areas in the primary forest, most of which were less than about 100 m^2 in area. In order to clearly distinguish canopy gaps from closed-canopy areas, we defined canopy gaps as open canopy areas around which we could find a fallen tree. We set up 20 quadrats in canopy gaps at the forest floor (the gap sites); each quadrat was 5 m × 5 m. We also placed a quadrat of the same size about 10 m away from each gap quadrat in a closed-canopy area (the non-gap sites). At each quadrat (a total of 40), all plants taller than 50 cm and with leaves below a height of 2 m above the ground were examined up to a height of 2 m above the ground. We recorded when ants attended EFNs or hemipterans, and collected samples from these plants. All of the collected specimens of ants, plants, and hemipterans (except for a very small number of lost samples) were sorted and identified to species, morphospecies, or higher taxonomic level (genus or family).

We checked the ant-attended parts of the plants for the presence of “shelters” that had been constructed by ants. The ant-attended leaves or shoots were also categorized by color, size, and freshness (hard or soft tissure).

We defined “the frequency of ant–hemiptera interaction” and “the frequency of ant–EFN interaction” as the proportion of plants in which we found ant–hemiptera or ant–EFN interactions (i.e., the number of plants exhibiting such interactions divided by the total number of investigated plants for each quadrat).

We also found some myrmecophytes in the study area. Myrmecophytes prepare special nesting space (domatia) for their obligate ant partners and often provide the ants with food. Most of the obligate mutualisms between ants and myrmecophytes involve scale insects (Hemiptera: Sternorrhyncha: Coccoidea), and ants live only on the plants and feed exclusively on plant food bodies and honeydew excreted by sap-sucking homopterans in the domatia. The ants, in return, protect the plant from herbivores and vines

(Itioka et al. 2000; Fiala and Maschwitz 1990). There is thus a strong relationship between ants, hemipterans, and plants. In this study, we treated the myrmecophytes separately as an independent symbiotic system.

The plants were categorized into three phases of leaf opening, (1) leaves not yet opened, (2) leaves just after opening, (3) plants having both previous categories present. Plants in the third category are defined as having a continuous succession of new leaves available.

Evaluation of light intensity

To assess light intensity, hemispherical photographs were taken with a Nikon camera (Nikon Co., Tokyo, Japan) equipped with an 8-mm fisheye lens at each quadrat. These digital photographs were analyzed using Gap Light Analyzer Version 2.0 (GLA; Frazer et al. 1999). We estimated the amount of direct solar radiation intensity per day (MJ m^{-2} d^{-1}) at the floor.

Results

Hemipterans and EFN attended by ants

As expected, the frequencies of both ant–hemipteran and ant–EFN interactions were significantly higher at gap sites than at non-gap sites (Wilcoxon signed-rank test, $P = 0.0021$; 0.0026, respectively). In addition, the species richness of ants, hemipterans, and plants were also higher at canopy gap sites than at non-gap sites. The frequency of myrmecophytes was also higher at gap sites (Wilcoxon signed-rank test, $P = 0.0067$).

Compared with non-gap sites, hemipterans being attended by ants tended to use younger parts of the plants in canopy gaps (Fisher's exact test, $P = 0.017$). Conversely, the proportion of hemipterans around which ants formed a shelter was significantly higher at non-gap sites than at gap sites (Fisher's exact test, $P < 0.001$).

When we classified hemipterans by their movement ability, the groups with relatively good flying ability (e.g., Membracidae and Cicadellidae) tended to appear in gap sites, and other groups with poor flying ability (e.g., Coccoidea and Aphididae) used non-gap sites (Fisher's exact test, $P = 0.016$).

We detected ant–EFN interactions at the young part of plants (e.g., new leaves) (binomial test, $P < 0.001$; young parts = 26, older parts = 4).

Ant–hemipteran–plant interactions

We detected no pattern of specific ants using specific plants or hemipterans, or vice versa. Of 17 ant species found in at least two trophobioses, none was restricted to a single plant family.

Light intensity and new leaf availability of plants

Gap quadrats have a significantly higher ratio of plants with a continuous availability of new leaves compared with non-gap quadrats (Wilcoxon signed-rank test, $P = 0.0018$). Furthermore, this proportion was significantly correlated with the light intensity at the sampling location (Spearman’s rank correlation coefficient = 0.41, $R^2 = 0.12$, $P = 0.0093$). The numbers of ant–hemipteran and ant–EFN interactions per quadrat both increased significantly with the number of plants that have a continuous availability of new leaves.

Discussion

The effect of canopy gaps on plants has been emphasized for a long time (Lawton and Putz 1988; Denslow

1995; Hubbell et al. 1999; Galhidy et al. 2006). In this study, we revealed that there is a higher proportion of ant-attended plants in canopy gaps, as well as a higher number of ants species in canopy gaps, compared with in closed-canopy areas. Since numerous arboreal ants in tropical rain forests feed mainly as herbivores or on insect exudates (e.g., from hemipterans; Davidson et al. 2003), canopy gaps may be an important factor in sustaining the biomass and diversity of ants in tropical rain forests. Moreover, plants and hemipterans that offer liquid food to ants were also diverse in canopy gaps. These results indicate that the canopy gap environment influences not only plants but also ant–hemipteran–plant interactions.

In addition, these effects are varied and not limited to certain species or interactions because no patterns of specificity were found. Furthermore, certain ant species used EFNs and hemipterans simultaneously on the same plants. This plasticity will enable ants to use nearby plants both directly and indirectly. It is likely that the complex web containing potential species replacements leads to the maintenance of species diversity in canopy gaps. Plants in canopy gaps act as a food supply not only for ants that live in the gaps but also for those in the surrounding closed-canopy areas because most of the ant species found in the non-gap areas were also found at the canopy gaps. Levey (1990) described canopy gaps as keystone habitats for frugivores because of the availability of abundant food resources. Similarly, we can say that canopy gaps also work as essential space—as Levey put it, “keystone habitats”—for ants and ant–hemipteran–plant interactions.

Judging from our results, we speculate that canopy gaps have a more multifarious influence than previously thought. They may contribute to increasing and maintaining diversity for various groups, especially in the tropical forest. We expect that future studies of insects (besides ants and hemipterans), other arthropods, and mammals and of other ecosystems will demonstrate the great effect of canopy gaps.

Ant-attended hemipterans and EFNs tended to be present on the younger parts of plants. It is likely that the increased amount of sunlight reaching the forest floor in canopy gaps causes the increased number of new leaves and brought about the increase in ant–hemipteran–plant interactions.

The occurrence of canopy gaps is unpredictable, and they do not last long. The fact that hemipterans with relatively good flying ability appeared in canopy gaps shows that the ability to move enables hemipterans to become established on the productive plants in canopy gaps. Conversely, closed-canopy areas are stable compared with canopy gaps. Therefore, hemipterans with limited migration ability found in the shaded understory might put a greater value on a stable relationship with ants rather than on the increased resource availability in gaps. The flight ability of hemipterans may thus represent an adaptation to their preferred environment (i.e., gap vs. non-gap). Our results show that the variations of plants caused by canopy gaps can affect fauna or mutualisms through the process of a nutritional cascade. A slight environmental difference might produce variation in the quantity and quality of interactions. MacArthur et al. (1966) suggested that, because of the higher structural heterogeneity in tropical forests than in the temperate areas, tropical individuals should have higher habitat specificity than individuals in the temperate zone. It was observed in our study that canopy gaps work as keystone habitats for ants and ant–hemipteran–plant interactions, and adaptations to different environments (i.e., gap vs. non-gap) are also important for maintenance of species diversity. Our results should provide insights into the maintenance of species diversity in the tropical rain forest.

Acknowledgments

We are grateful to Ms. L. Chong (Forest Research Centre Sarawak) and Mr. J. J. Kendawang (Forest Department Sarawak) for providing the study site and kind assistance. We thank Prof. T. Nakashizuka (Tohoku University) and Dr. M. Ichikawa (Research Institute for Humanity and Nature) for providing opportunities for this study.

References

Beck H, Gaines MS, Hines JE, Nichols JD (2004) Comparative dynamics of small mammal populations in treefall gaps and surrounding understorey within Amazonian rainforest. Oikos 106:27–38

Brylinski M, Mann KH (1973) An analysis of factors governing productivity in lakes and reservoirs. Limn. Ocean. 18:1–14

Cole JJ, Findlay S, Pace ML (1988) Bacterial production in fresh and saltwater ecosystems: a cross systems overview. Mar. Ecol. Prog. Ser. 43:1–10

Coley PD (1983) Herbivory and defensive characteristics of tree species in a lowland tropical forest. Ecol. Mon. 53:209–233

Davidson DW (1997) The role of resource imbalances in the evolutionary ecology of tropical arboreal ants. Biol. J. Linn. Soc. 61:153–181

Davidson DW, Cook SC, Snelling RR, Chua TH (2003) Explaining the abundance of ants in lowland tropical rainforest canopies. Science 300:969–972

Denslow JS (1995) Disturbance and diversity in tropical rain forests - the density effect. Ecol. Appl. 5:962–968

Fiala B, Maschwitz U (1990) Studies On the South East-Asian ant-plant association Crematogaster-Borneensis Macaranga - adaptations of the ant partner. Insect. Soc. 37:212–231

Frazer GW, Canham CD, Lertzman KP (1999) Gap Light Analyzer (GLA), Version 2.0:Imaging Software to Extract Canopy Structure and Gap Light Transmission Indices from True-Colour Fisheye Photographs, Users Manual and Program Documentation. Simon Fraser University, Burnaby, British Columbia and Institute of Ecosystem Studies, NY

Galhidy L, Mihok B, Hagyo A, Rajkai K, Standovar T (2006) Effects of gap size and associated changes in light and soil moisture on the understorey vegetation of a Hungarian beech forest. Plant Ecol. 183:133–145

Hölldobler B, Wilson EO (1990) The Ants. Harvard University Press, Cambridge

Hubbell SP, Foster RB, O'Brien ST, Harms KE, Condit R, Wechsler B, Wright SJ, Lao SL (1999) Light-gap disturbances, recruitment limitation, and tree diversity in a neotropical forest. Science 283:554–557

Itioka T, Nomura M, Inui Y, Itino T, Inoue T (2000) Difference in intensity of ant defense among three species of Macaranga myrmecophytes in a southeast Asian dipterocarp forest. Biotropica 32:318–326

Kato M, Inoue T, Hamid AA, Nagamitsu T, Merdek MB, Nona AR, Itino T, Yamane S, Yumoto T (1995) Seasonality and vertical structure of light-attracted insect communities in a dipterocarp forest in Sarawak. Res. Pop. Ecol. 37:59–79

Lawton RO, Putz FE (1988) Natural disturbance and gap-phase regeneration in a wind-exposed tropical cloud forest. Ecology 69:764–777

Levey DJ (1988) Tropical wet forest treefall gaps and distributions of understory birds and plants. Ecology 69:1076–1089

Levey DJ (1990) Habitat-dependent fruiting behavior of an understorey tree, Miconia-Centrodesma, and tropical treefall gaps as keystone habitats for frugivores in Costa-Rica. J. Trop. Ecol. 6:409–420

MacArthur RH, Recher H, Cody M (1966) On the relationship between habitat selection and species diversity. Am. Nat. 100:319–332

Nagamasu H, Momose K (1997) Flora of Lambir Hills National Park, Sarawak, with special reference to the Canopy Biology Plot. In: Canopy biology program in Sarawak. II. General flowering of tropical forests in Sarawak (eds. Inoue T and Hamid AA) pp. 20–67. Center for Ecological Research, Kyoto University, Otsu

Tobin JE (1995) Ecology and diversity of tropical forest canopy ants. In: Forest canopies (eds. Lowman MD and Nadkarni N) pp. 129–147. Academic Press, New York

Yamane S, Nona AR (1994) Ants from Lambir Hills National Park. In: Canopy biology program in Sarawak. I. Plant reproductive systems and animal seasonal dynamics (eds. Inoue T and Hamid AA) pp. 223–226, Kyoto

Predator Avoidance Effects of Southeast Asian Danaid Butterflies in the Wild

Taizo Tzuchiya, Takao Itioka
Graduate School of Human and Environmental Studies, Kyoto University

Chemical defenses, a typical butterfly defense strategy, have been well studied (Turner 1984), especially for danaid butterflies. The monarch butterfly, *Danaus plexippus* (Danaidae), which ranges mainly in North and Central America, is the best studied species in this family. Monarchs become unpalatable to birds, the major predators of adult butterflies (Edmunds 1974), when their larvae feed on poisonous milkweed (*Asclepias* spp., Asclepiadaceae) or other plants that contain cardiac glycosides (cardenolides). Birds learn to reject monarch butterflies after they have eaten one and vomited (Brower 1958; Brower 1969). Not all of the monarch butterfly’s food plants contain cardenolides, however, and birds repeatedly ate monarchs reared on plants that lacked cardenolides (e.g., *Asclepias syriaca*, *A. tuberosa*, and *A. incarnata*). These studies indicate that the palatability of monarch butterflies was directly related to the species of plant ingested by the larvae (Brower 1969).

Other studies have raised doubts about predator avoidance of danaid butterflies in Southeast Asia. Several species of the danaid butterflies in this area are polyphagous and have been observed feeding on a wide range of seven plant families (Robinson et al. 2001), and there is no evidence of cardenolides in these plants. Furthermore, chemical analyses have shown that *Euploea core* (Danaidae), which is one of the most common species of danaid butterfly in this area, stores emetic cardenolides only as larvae and not as pupae and adults when reared on *Nerium oleander* (Apocynaceae) (Malcolm and Rothschild 1983).

We found no field studies of predator avoidance of danaid butterflies in Southeast Asia except for Ohsaki (1995)’s examination of predator avoidance of danaid butterflies. He used “beak marks” (certain types of wing damage that represent an unsuccessful attack by birds; Johki 1985) on butterfly wings as an indicator of predation pressure by birds. A high frequency of beak-marked species should reflect a high attack rate by birds (Edmund 1974). Ohsaki showed that the proportion of individuals with beak marks in the Danaidae family was significantly lower than it was for other nonpoisonous families of butterflies. However, beak-mark frequency is only indirect evidence of bird predation pressure (Edmunds 1974). Thus, we need to evaluate avoidance effects with a more direct method.

Our objective was to investigate the avoidance effects of Southeast Asian danaid butterflies in the wild. We evaluated how much these butterflies were avoided by predators compared with putative nonpoisonous butterflies (mostly Nymphalidae, Satyridae, Pieridae, and Papilionidae) in a tropical rainforest in Lambir National Park (4°20'N, 113°50'E), Sarawak, Malaysia. First, we regularly collected the butterflies and scored them for the presence of beak marks on their wings. Then, as a more direct method of observation, we conducted field presentation experiments employing the butterfly specimens.

Materials and Methods

Beak-mark rate

To determine the proportion of individuals with beak marks (the beak-mark rate), we collected butterflies on an approximate 3-km transect across the inside and outside of a primeval forest in Lambir National Park from 2004 to 2006. We walked the transect in both directions twice a week and captured butterflies that were within 3 m on either side and 5 m in the front with a net (for details on the method, see Pollard 1975). We then recorded the presence or absence of beak marks on the butterfly's wings (for details on the method, see Johki 1985).

We compared the beak-mark rate of danaid butterflies with those of butterflies from four nonpoisonous families (Nymphalidae, Satyridae, Pieridae, and Papilionidae). We omitted some genera (e.g., *Delias, Cethosia, Elymnias,* and *Chilasa*) from this analysis because most of them are thought to be poisonous or to mimic other unpalatable butterflies.

Field presentation experiment

For the field presentation experiment, we selected four abundant species of danaid butterfly (*Euploea diocletianus*, *E. mulciber*, *E. crameri,* and *Parantica aspasia*) and two species of nymphalid butterfly (*Lexias pardalis* and *Tanaecia munda*), which had roughly the same wing size (Otsuka 1998) and sometimes shared the same habitat (personal observation). Food plants of these butterflies at this study site are unknown, but there are several reports that *Euploea* and *Parantica* in Southest Asia feed mainly on Asclepiadaceae, Apocynaceae, and Moraceae (many species in these families are inferred to be poisonous), whereas *Tanaecia* and *Lexias* probably feed on plants that are not poisonous (e.g., Lecythidaceae and Hypericaceae, respectively) (Robinson et al. 2006).

We carried out a field presentation experiment during the day in March, May-June, and August-October 2003 and April 2004. We caught the butterflies with a net and stored them in a freezer. One day before the experiments, we took them out of the freezer and then unfolded the specimen's wings at about a 90° angle and fixed them in order to make the specimens imitate the natural state of a butterfly's wings.

We paired one specimen from each of the four Danaidae species with one from each of the two Nymphalidae species to make eight different types of butterfly pairs. We selected a pair from among the eight and tied the thorax of each butterfly specimen with a fishing line. The line was attached to a fishing rod, and two rods were used for the presentation of one pair.

We simultaneously passed the ends of lines holding the specimen pair from the canopy walkway to the subcanopy layer (roughly 10 m below). We defined a wild bird's response as an "approach" when a bird came within 2 m of either specimen. We recorded the species of the approached butterfly specimen and the bird whenever possible. If no bird approached the offered specimens within 20 minutes, we removed them and presented them again at another point. We compared the approach frequency between the danaid and nymphalid butterfly specimens for each of the eight pairs.

Results

Beak-mark rate

Figure 1 shows the mean beak-mark rate of each family. The beak-mark rate of family Danaidae was significantly lower than those of the Satyridae, Nymphalidae, and Pieridae families (Ryan's multiple-comparison method, $P < 0.05$ in each case) but not for Papilionidae. In contrast, we found no significant differences among the four nonpoisonous families ($P > 0.05$ in each case).

Field presentation experiment

In total, 399 pair-wise presentations were conducted, and 53 specimens were approached by birds. All specimens approached were Nymphalidae butterflies—no Danaidae butterflies were approached. The approach responses of birds were significantly less frequent to Danaidae specimens than to Nymphalidae ones for five of the eight pairs (Fisher's exact test, $P<0.01$) (Fig. 2). The total approach frequency was insufficient for statistical analyses for the other three pairs. In some cases, we could identify the bird species approaching the offered specimen, but precise determination was often difficult. We recorded birds from at least three families (Pycnonotidae, Timaliidae, and Irenidae).

Discussion

Beak marks have been interpreted as evidence of active escape by the prey (Edmunds 1974). If danaid butterflies are unpalatable, then birds that have sampled them may learn to avoid them. Thus, there should be fewer attacks and beak marks on danaid butterflies than on other butterflies. The low beak-mark rate we observed for danaid butterflies suggests that they were attacked less frequently by birds than were other putative nonpoisonous butterflies at this study area. These results are in agreement with those found by Ohsaki's (1995) study conducted in Sabah, Malaysia. However, beak-mark rates in natural populations of butterflies are not only affected by palatability (Edmunds 1974). Thus, we must be careful when interpreting these results.

The low approach frequency of birds to the offered danaid specimens more directly showed that they were avoided by some birds in the wild. Among the birds, most species of Pycnonotidae and some of Timaliidae are known to forage on some insects on trees (Smythies 1999). Therefore, we interpreted the bird's approach behavior as an attempt to prey on the offered specimen. Indeed, the bird caught the specimen in some cases. In many of the cases, the bird most likely noticed the fishing line just before attacking the butterfly and left.

Our results suggest that some insectivorous birds were more reluctant to attack danaid butterflies than other butterflies at this study site. This rejection may be due to unpalatability caused by the presence of some chemical substances in the Danaid butterflies.

Acknowledgments

We are grateful to Mr. Joseph Jawa Kendawang and Ms. Lucy Chong of the Sarawak Forest Corporation for permitting access to the study site and supporting our research in Sarawak.

References

Brower JVZ (1958) Experimental studies of mimicry in some North American butterflies. I. The monarch, *Danaus plexippus*, and viceroy, *Limenitis archippus*, Evolution 12:32–47

Brower LP (1969) Ecological chemistry, Scientific American 220:22–29

Edmunds M (1974) Significance of beak marks on butterfly wings, Oikos 25:117–118

Johki Y (1985) Wing damages of butterflies and bird's attacks, Tyo to Ga 35:202–207

Malcolm S, Rothschild M (1983) A danaid mullerian mimic, *Euploea core amymone* (Cramer) lacking cardenolides in the pupal and adult stages. Biological Journal of the Linnean Society 19:27–33

Ohsaki N (1995) Preferential predation of female butterflies and the evolution of batesian mimicry, Nature 378:173–175

Otsuka K (1988) *Butterflies of Borneo*, Tobishima Corporation, Tokyo.

Pollard (1975) A method for assessing changes in the abundance of butterflies, Biological Conservation 12:115–134

Robinson GS, Ackery PR, Kitching IJ, Beccaloni GW, Hernandez LM (2001) *Hostplants of the moth and butterfly caterpillars of the Oriental Region*. Southdene Sdn Bhd, Kuala Lumpur

Smythies BE (1999) *The birds of Borneo*, Natural history publications (Borneo) Sdn. Bhd. Kota Kinabalu

Turner JRG (1984) Mimicry: the palatability spectrum and its consequences, In *The Biology of Butterflies*, RI Vane-Wright and PR Ackery eds., Academic Press, London

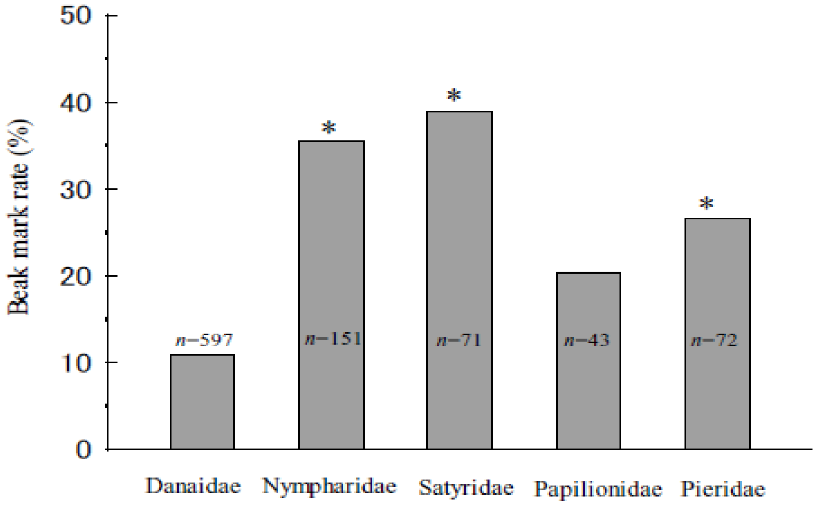


Fig. 1. Percentage of butterflies with beak marks in each families.
* indicates the beak mark rate differes siginigicntly from its Danaidae (Ryan's multiple comparison test $P<0.05$).

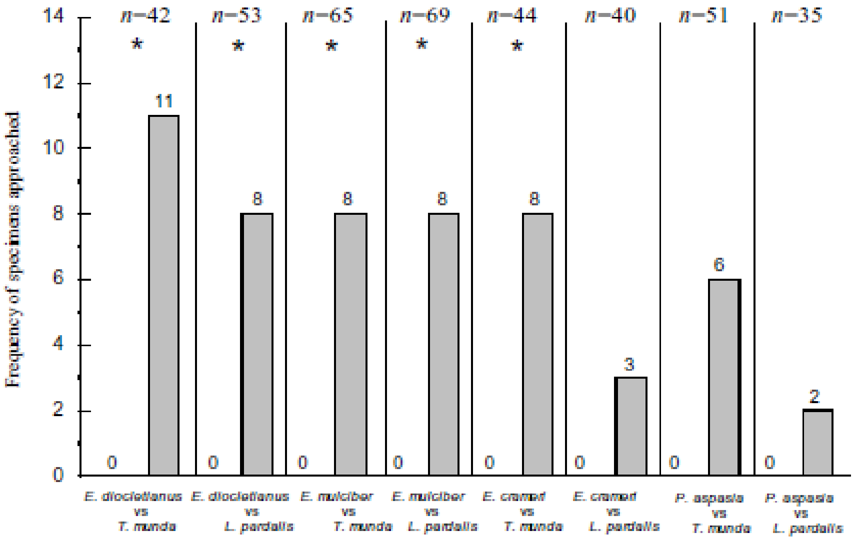


Fig. 2. Number of butterfly speciemens attacked in each pair wise presentation experiment. * indicates significant difference (Fisher's exact test $P<0.01$).

The Morphological and Ecological Characteristics of *Arhopala* Species (Lycaenidae) Utilizing *Macaranga* Species (Euphorbiaceae) as Host Plants in a Bornean Dipterocarp Forest

Tadahiro Okubo, Takao Itioka
Environment Graduate School of Human and Environmental Studies, Kyoto University,
Yoshidanihonmatsu, Sakyo-ku, Kyoto 606-8501, Japan

Introduction

The Asian butterfly genus *Arhopala* (Lycaenidae) includes 160 species that are distributed from India to Australia. The *amphimuta* subgroup of this genus is known to utilize *Macaranga* species (Euphorbiaceae) as its host plants (Maschwitz et al. 1984; Megens et al. 2005). Genus *Macaranga* includes more than 20 species of myrmecophytes (Whitmore 1969; Quek et al. 2004). These myrmecophytic species develop mutualistic relationships with species-specific specialist ants of the genera *Crematogaster* or *Camponotus* (Fiala and Maschwitz 1991, 1992). These plants provide nesting space for ants in the internodes and produce food bodies in the leaves and stipules that provide a food resource for the ants. The symbiotic ants exclude herbivores by responding to volatile chemicals from injured tissues of the host plant; they quickly aggregate at the injured points, where they attack the herbivores.

Arhopala larvae could feed on such myrmecophytic *Macaranga* if they are capable of evading the aggressive ants. However, studies of the utilization of *Macaranga* as a host plant by *Arhopala* are limited. In the Malayan peninsula, only three *Macaranga* species have been investigated. Each of these species serves as a host for the larvae of one *Arhopala* species, and symbiotic ants do not appear to attack the *Arhopala* larvae (Maschwitz et al. 1984). There is wide variation in the intensity of the mutual dependency in ant–*Macaranga* interactions, ranging from non-myrmecophytes to facultative, transitional, and obligate myrmecophytes (Fiala and Maschwitz 1990; Fiala et al. 1994). In addition, the defenses of *Macaranga* include defense by ants and chemical or physical defenses (non-ant defenses). The balance between ant and non-ant defenses also differs among *Macaranga* species (Itioka et al. 2000; Nomura et al. 2000; Itioka 2005).

Each *Arhopala* species that utilizes *Macaranga* may overcome one or more components of the variation in anti-herbivore defenses by *Macaranga*. However, the relationship between *Macaranga* and *Arhopala* has not been investigated, and as a result, the survival strategy of each *Arhopala* species that utilizes *Macaranga* has not yet been revealed. In the present study, we investigated the relationships between *Macaranga* and *Arhopala* in Borneo, where the highest species richness of *Macaranga* is found. In addition, we describe the morphology and ecology of the *Arhopala* larvae, and especially the interaction between the larvae and symbiotic ants.

Methods

Study site

Our study was conducted in Lambir Hills National Park, Sarawak, Malaysia (4˚2'N, 113˚2'E, 150 to 200m a.s.l.), from the end of May 2006 to the beginning of March 2007. This park is covered primarily by lowland mixed dipterocarp forest. More than 16 *Macaranga* species, including at least 11 myrmecophytic species, occur in the park.

Sampling and rearing

We investigated 11 myrmecophytic and 5 non-myrmecophytic *Macaranga* species to obtain *Arhopala* eggs and larvae. During this collection, we recorded the parts of the plant on which the larvae fed, the location of the larvae, ant behavior in response to the larvae, and the number of attendant ants. The collected eggs and larvae were taken to the laboratory on their host plants for rearing and observation. In the laboratory, the eggs and larvae were maintained in plastic boxes with their host plants until they reached the adult stage. We observed any myrmecophilic organs on the larvae and recorded. The host plants were maintained by cutting the stems and inserting them in sponges used in floral arrangements (i.e., in floral oases).

Results

In total, we recovered 18 eggs and 93 larvae of *Arhopala* from four myrmecophytic and two non-myrmecophytic *Macaranga* species. In the myrmecophytic plants (*M. trachyphylla*, *M. bancana*, *M. beccariana*, and *M. hosei*), each plant hosted the larvae of a single *Arhopala* species; two closely related species (*M. trachyphylla* and *M. bancana*) hosted conspecific *Arhopala*. Two non-myrmecophytic plants (*M. gigantea* and *Macaranga* sp. A) were also utilized by conspecific *Arhopala*. The morphological and ecological characteristics differed among the *Arhopala* species (Table 1).

1. ***Macaranga trachyphylla, M. bancana – Arhopala amphimuta***

Arhopala amphimuta utilized *M. trachyphylla* and *M. bancana* (both myrmecophytes) as its host plant. These two species are closely related. One or two eggs covered with many warts were laid on the underside of fresh leaves (Fig. 1). The emerging light-green larvae rested on the underside of fresh leaves (Fig. 2). Some leaves of *M. trachyphylla* and *M. bancana* developed a red color on their underside. When larvae fed on these red leaves, they tended to develop a red-brown band in the middle part of the green body of the middle-instar larvae (Fig. 3). Larvae that fed on green leaves did not develop this band. All larvae developed myrmecophilic organs (a dorsal nectary organ and tentacle organs; Fig. 4). Symbiotic ants of *Macaranga* did not attack the larvae and instead appeared to tend them (Figs. 2–4). The number of ants that tended a last-instar larva averaged 6.8 ± 0.97 (mean ± SE, N = 5). The pupal body color was similar to that of the stipules of the host plant (Fig. 5). A parasitic fly emerged from the body of a prepupa, and an ichneumonid fly emerged from the body of a pupa. 24% of the collected larvae were parasitized by either a parasitic fly or an ichneumonid fly.

2. ***Macaranga beccariana – Arhopala zylda***

Arhopala zylda utilized *M. beccariana*, a myrmecophyte host plant. The whitish-green larvae rested on the underside of fresh leaves, and their body color was similar to that of these fresh leaves (Figs. 6–8). The larvae only developed tentacle organs and did not exhibit a dorsal nectary organ. Symbiotic ants of

Macaranga did not tend these larvae, but they also did not attack the larvae. The pupal body color was similar to that of fresh leaves of the host plants (Fig. 9). 23% of the collected larvae were parasitized by either a parasitic fly or an ichneumonid fly.

3. ***Macaranga hosei – Arhopala dajagaka***

Arhopala dajagaka utilized *M. hosei*, a myrmecophyte host plant. One or two eggs covered with many warts were laid on the underside of fresh leaves (Fig. 10). The larvae had a greenish-yellow color that is similar to that of fresh leaves of the host plant (Figs. 11–13). This species developed two myrmecophilic organs (a dorsal nectary organ and tentacle organs). The larvae of this species appeared to exude more honeydew from their dorsal nectary organ than the larvae of *A. amphimuta*. The number of ants that were tending a last-instar larva averaged 17.5 ± 2.72 (mean ± SE, $N = 4$). The pupal body color was similar to that of the fresh leaves of the host plants (Fig. 14). 25% of the collected larvae were parasitized by either a parasitic fly or an ichneumonid fly.

4. ***Macaranga gigantea, Macaranga* sp. A – *Arhopala major***

Arhopala major utilized *M. gigantea* and *Macaranga* sp. A, both non-myrmecophytes, as its host plant. Only one larva was discovered on *Macaranga* sp. A, thus most of the larvae were discovered on *M. gigantea*, which has very large leaves. One to three eggs covered with many warts were laid on the underside of fresh leaves (Fig. 15). The larvae developed two myrmecophilic organs (a dorsal nectary organ and tentacle organs). Their body color ranged from light green to pale yellow and was similar to that of fresh leaves of the host plant (Figs. 16–18). Many fresh leaves had feeding damage caused by these larvae, but the larvae were rarely discovered on fresh leaves. This is because they usually rested inside stipules during the day (Fig. 17). The pupal body color was very similar to that of the stipules of the host plant (Fig. 19). A braconid wasp emerged from the body of a middle-instar larva, and a parasitic fly emerged from the body of a prepupa. 37% of the collected larvae were parasitized by either a parasitic fly or a braconid wasp.

Discussion

In the Malayan peninsula, three *Arhopala* species have been reported to utilize *Macaranga* species (Maschwitz et al. 1984). These species formed three associations: *A. amphimuta–M. triloba, A. moolaiana–M. hulletti*, and *A. zylda–M. hypoleuca*. In the present study (in Borneo), *A. amphimuta* utilized *M. trachyphylla* and *M. bancana*, which are closely related to *M. triloba*. *M. triloba* was not found in Lambir Hills National Park. *Arhopala zylda* utilized *M. beccariana*, which is closely related to *M. hypoleuca*, but *M. hypoleuca* was not utilized by larvae of *A. zylda* in Lambir Hills National Park. The relationship between *Macaranga* and *Arhopala* species may thus exhibit a certain degree of species specificity. The larvae of *A. moolaiana* were not found on *Macaranga* in the present study, but many adults of this species were captured. This suggests that *A. moolaiana* larvae will be found on other *Macaranga* species in the Lambir Hills National Park.

The majority of lycaenids develop associations with ants that can be either facultative or obligate and that range from mutualism to parasitism (Pierce et al. 2002). Ant association has exerted a strong selection pressure on lycaenid larval shape. Thus, the majority of lycaenid larvae develop myrmecophilic organs to protect themselves from ant attack. In the present study, the larvae of *A. amphimuta* and *A. dajagaka* developed two myrmecophilic organs and were tended by symbiotic ants on myrmecophytic *Macaranga*; however, *A. zylda* did not develop a dorsal nectary organ and was not tended by ants on myrmecophytic *Macaranga* (Table 1). Thus, larvae may be tended by the ants because they are attracted by honeydew secreted from the dorsal nectary organ. In addition, the difference in the number of ants found attending a last-instar *Arhopala* larva between *A. amphimuta* and *A. dajagaka* is likely to result from differences in the amount of honeydew provided for the ants.

Despite their lack of a nectary, the larvae of *A. zylda* were not attacked by the ants. These larvae may have evolved a chemical mimicry to penetrate symbiotic ant–*Macaranga* partnerships. The location and parasitoids of the larvae differed between the myrmecophytic and non-myrmecophytic *Macaranga* (Table 1). The larvae of *A. amphimuta*, *A. dajagaka*, and *A. zylda* on myrmecophytic *Macaranga* may protect themselves from braconid wasps by coexisting with the ants, which may defend them from the wasps. On the other hand, the larvae of *A. major* on non-myrmecophytic *Macaranga* may protect themselves from ichneumonid flies by hiding in host stipules. The larvae and pupae of the four *Arhopala* species tended to have coloration and shape similar to those of the fresh leaves or stipules that each species utilizes. The survival strategy of each *Arhopala* species may thus correspond to the specific ant–*Macaranga* symbiosis. The differences in morphological and ecological characteristics among the *Arhopala* species are likely due to differences in the anti-herbivore defense strategy adopted by the *Macaranga* species.

Acknowledgments

We thank Ms. L. Chong and Mr. J. J. Kendawang (Sarawak Forest Corporation) and Prof. T. Nakashizuka (Tohoku University) for providing us with the opportunity to carry out this study. We also thank Dr. K. Yago (The University of Tokyo) for valuable comments on butterfly identification.

References

Fiala B, Grunsky H, Maschwitz U, Linsenmair KE (1994) Diversity of ant-plant interactions: protective efficacy in *Macaranga* species with different degrees of ant association. Oecologia 97:186-192

Fiala B, Maschwitz U (1990) Studies on the South East Asian ant-plant association *Crematogaster borneensis* / *Macaranga*: Adaptations of the ant partner. Ins Soc 37:212-231

Fiala B, Maschwitz U (1991) Extrafloral nectaries in the genus *Macaranga* (Euphorbiaceae) in Malaysia: Comparative studies of their possible significance as predispositions for myrmecophytism. Biol J Linn Soc 44:287-306

Fiala B, Maschwitz U (1992) Food bodies and their significance for obligate ant-association in three genus *Macaranga* (Euphorbiaceae). Biol J Linn Soc 110:61-75

Itioka T (2005) Diversity of anti-herbivore defenses in *Macaranga*. In: Roubik DW, Sakai S, Karim AAH (eds) Pollination ecology and the rain forest: Sarawak studies. Springer, New York, pp 158-171

Itioka T, Nomura M, Inui Y, Itino T, Inoue T (2000) Difference in intensity of ant defense among three species of *Macaranga* myrmecophytes in a Southeast Asia dipterocarp forest. Biotropica 32:318-326

Maschwitz U, Schroth M, Hanel H, Pong TY (1984) Lycaenids parasitizing symbiotic plant-ant partnerships. Oecologia 64:78-80

Megens HJ, Jong RD, Fiedler K (2005) Phylogenetic patterns in larval host plant and ant association of Indo-Australian Arhopalini butterflies (Lycaenidae: Theclinae). Biol J Linn Soc 84:225-241

Nomura M, Itioka T, Itino T (2000) Variations in abiotic defense within myrmecophytic and non-myrmecophytic species of *Macaranga* in a Bornean dipterocarp forest. Ecol Res 15:1-11

Pierce NE, Brady MF, Heath A, Lohman DJ, Mathew J, Rand B, Travassos MA (2002) The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). Annu Rev Entomol 47:733-771

Quek SP, Davies SJ, Itioka T, Pierce NE (2004) Codiversification in an ant-plant mutualism: stem texture and the evolution of host use in *Crematogaster* (Formicidae: Myrmicinae) inhabitants of *Macaranga* (Euphorbiaceae). Evolution 58:554-570

Whitmore TC (1969) First thoughts on evolution in Malayan *Macaranga*. Biol J Linn Soc 1:223-231

Figs.1-5. *Arhopala amphimuta*. 1. Egg of *A. amphimuta* after hatching. 2. First instar larva of *A. amphimuta* underside of fresh leaf. 3. Middle instar larva of *A. amphimuta* attended by some symbiotic ants of *M. trachyphylla*. 4. Last instar larva of *A. amphimuta*. **A**: a dosal nectary organ. **B**: tentacle organs. 5. Pupa of *A. amphimuta*.

Figs.6-9. *Arhopala zylda*. 6. First instar larva of *A. zylda* underside of fresh leaf. 7. Middle instar larva of *A. zylda* on stem. 8. Last instar larva of *A. zylda*. 9. Pupa of *A. zylda* underside of fresh leaf.

Figs.10-14. *Arhopala dajagaka*. 10. Egg of *A. dajagaka* underside of fresh leaf. 11. First instar larva of *A. dajagaka* underside of fresh leaf. 12. Middle instar larva of *A. dajagaka* on leaf stem attended by some symbiotic ants of *M. hosei*. 13. Last instar larva of *A. dajagaka*. 14. Pupa of *A. dajagaka*.

Figs.15-19. *Arhopala major*. 15. Egg of *A. major* underside of fresh leaf. 16. Second instar larva of *A. major* underside of fresh leaf. 17. Middle instar larva of *A. major* inside of *M. gigantea* stipule. 18. Last instar larva of *A. major*. 14. Pupa of *A. major*.

Table 1. Summary of morphological and ecological characteristics of *Arhopala* larvae utilizing *Macaranga*

			Morphological character		**Ecological character**				
***Arhopala* species**	**Host plants**		**The body length of last instar larva**	**Myrmecophilic organs**	**Ant behavior to larvae**	**The number of attendant ants**	**Location of larvae**	**Feeding parts**	**Parasitoid of larvae**
A. amphimuta	**Ant plant**	***M. bancana* and *M. trachyphylla***	**16.5±0.5mm**	**Dorsal nectary organ and Tentacle organ**	**Attendance**	**6.8±0.97**	**Underside of fresh leaves**	**Fresh leaves**	**parasitic fly and ichneumon fly**
A. zylda	**Ant plant**	***M. beccariana***	**12.5±0.5mm**	**Tentacle organ**	**Non attack**	—	**Underside of fresh leaves**	**Fresh leaves and Food-bodies**	**parasitic fly and ichneumon fly**
A. dajagaka	**Ant plant**	***M. hosei***	**24.5±0.5mm**	**Dorsal nectary organ and Tentacle organ**	**Attendance**	**17.5±2.72**	**Underside of fresh leaves**	**Fresh leaves**	**parasitic fly and ichneumon fly**
A. major	**Non-ant plant**	***Macaranga sp.A* and *M. gigantea***	**20±1.0mm**	**Dorsal nectary organ and Tentacle organ**	—	—	**Inside of stipules**	**Fresh leaves**	**parasitic fly and braconid wasp**

Factors Controlling the Composition of Soil Microbial Communities in Tropical Forest Ecosystems

Rota Wagai, Nobuo Imai, Takami Satomura, Kanehiro Kitayama
Center for Ecological Research, Kyoto University

Introduction

Soil microbial community plays a fundamental role in ecosystem nutrient cycling through the mineralization of detritus organic matter (Chapin III *et al.*, 2002). In ecosystem models, the microbial mineralization process has been described as a function of environmental factors, and microbial community is regarded as a "black box". The role of different taxonomic or functional groups of microbes in ecosystem is therefore virtually unknown. Soil microbes appear to play particularly vital role in the tropics due to its unique geochemical condition. Tropical ecosystems are often maintained on highly weathered soils due to warm, humid climate regime and stability of land surface. Such tropical soils are dominated by reactive, small mineral particles down to tens of nano-meter in size (Birkeland, 1999). These minerals strongly influence biological processes at least by two geochemical reactions: (1) physical/chemical blocking of extracellular-enzyme activity and thus reduction in decomposition (nutrient mineralization) in consequence, and (2) sorption of phosphate, an essential nutrient for biota, and subsequent phosphorus limitation to plants and presumably to soil microorganisms. Observed high productivity and biomass of tropical forests, however, suggest that soil microbes adapted to these tropical soils efficiently mineralize nutrients by overcoming the geochemical constraints. We therefore tested the hypothesis that microbial community composition is controlled by soil mineral factors (e.g., geochemical condition and phosphorus limitation) as well as the availability of substrate (organic resource) using a series of tropical forest soils in Mt. Kinabalu. Based on the identified environmental factors that control microbial community in Mt. Kinabalu, we also speculated on the possible impact of logging on soil microbial community composition.

Methods

We studied soils on the eastern and southern slopes of Mt. Kinabalu (4095 m, 6^{o}05'N, 160^{o}33' E), developed on both acidic sedimentary and ultrabasic igneous parent materials, under the primary rain forests protected as the Kinabalu Park, Sabah, Malaysia. The six selected sites (at ca. 700, 1700, and 2700 m above sea level on the two rock types) are part of a long-term ecological study (Kitayama and Aiba, 2002). Briefly, the climate is humid tropical with weak influences of the Asiatic monsoon. A strong temperature gradient is present along the slope: mean annual air temperature (MAT) decreases with altitude at a mean lapse rate of 0.0055 ^{o}C m^{-1}, with <2 ^{o}C intra-annual variations. Mean annual rainfall is relatively constant (2300-2400 mm yr^{-1}) with elevation. Air and soil moisture generally increase with elevation due to more frequent cloud cover and less evapotranspiration at upper elevations.

We used phospholipid fatty acid (PLFA) biomarkers to assess shifts in microbial community composition. PLFA is a microbial cell membrane constituent and its chemical composition is unique for different broad taxonomic groups. At each site, soil samples were collected from O-horizon, 0-5, and 5-15 cm from three transects. Four to six cores were taken along each transect to make a composite. Soil profiles down to 1 meter depth (or to BC horizon) were described and samples are taken along the profiles at each site. All samples were brought back in cooler box and frozen within 6 hours after the sampling, followed by freeze-drying. The dried samples were sieved (2 mm), ground, and extracted for PLFA following Balser *et al*. (2005). Dried sample masses of 0.3-4.0 g were used for the extraction depending on the total organic carbon content of the sample. Fatty acids were extracted with 10:5:4 volumes of methanol, chloroform, and 0.1 M phosphate buffer (pH 7.0). Following purification, polar-lipid fraction (phospholipids) were isolated by silicic acid columns and subjected to saponification and methylation. Gas chromatograph was used to identify and quantify individual fatty acid methyl esters.

Results and Discussion

General trends

Total microbial PLFA concentration, a sensitive indicator of active microbial biomass, ranged 2.0-2.7 nmol g^{-1} in organic horizon (O-horizon) and 0.2-0.8 nmol g^{-1} in the surface mineral horizon (Fig. 1). These ranges are comparable to those in temperate forest and grassland soils. Corresponding to the variations in the concentration of soil organic matter (i.e. microbial substrate), the PLFA concentration of each broad taxonomic group as well as that of total microbial community decreased in the order: O-horizon > 0-5 cm > 5-15 cm. At 700 m and 2700 m sites where deeper soil samples were analyzed, bacteria showed clearer decline with increasing depth than fungi.

Elevation gradient

We considered two geochemical gradients (elevation and soil depth) to test above hypothesis as the abundance and chemistry of soil minerals predictably change along these gradients. Along the elevation gradient from 2700 m to 700 m, the ratio of gram-positive bacteria to gram-negative bacteria (G+:G- ratio) progressively increased in 0-5 cm mineral soils (Fig. 2) as well as O horizon (data not shown) in the sedimentary soils, while the ratio changed little in the ultrabasic soils. Along the same gradient, the ratio of fungi plus actinomycetes to bacteria consistently decreased at all three depths on both rock types. Fungi:bacteria ratio showed a similar yet less clear trend.

Relatively small standard deviations in these ratios (Fig. 2b) than those in each taxonomic group (Fig. 2a), together with above trends, suggest a significant shift in microbial community composition along the elevation gradient. Bacteria (esp. G+ ones) appeared to increase their relative abundance over fungi and actinomycetes in the surface soils under warmer climate regime where soil organic resources are lower in concentration and poorer in quality due to the protection by active mineral phases compared to the soils under cooler climate.

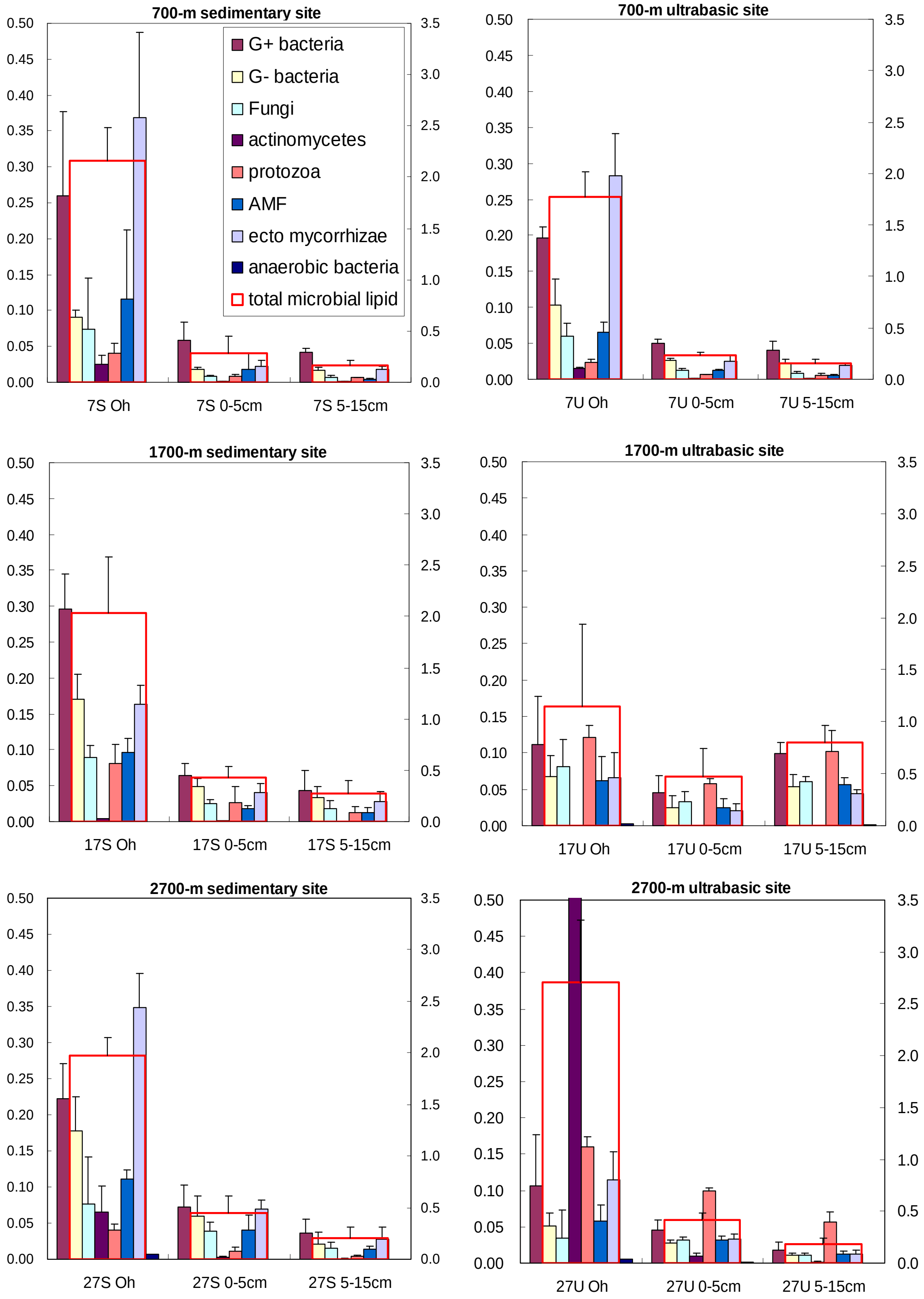


Figure. 1. PLFA concentrations of total active microbial biomass and broad taxonomic groups at each site and depth. N = 3 (error bar = SD). Left axis is PLFA concentrations of individual broad taxonomic group (nmol g^{-1}) and right axis is total microbial PLFA (nmol g^{-1}).

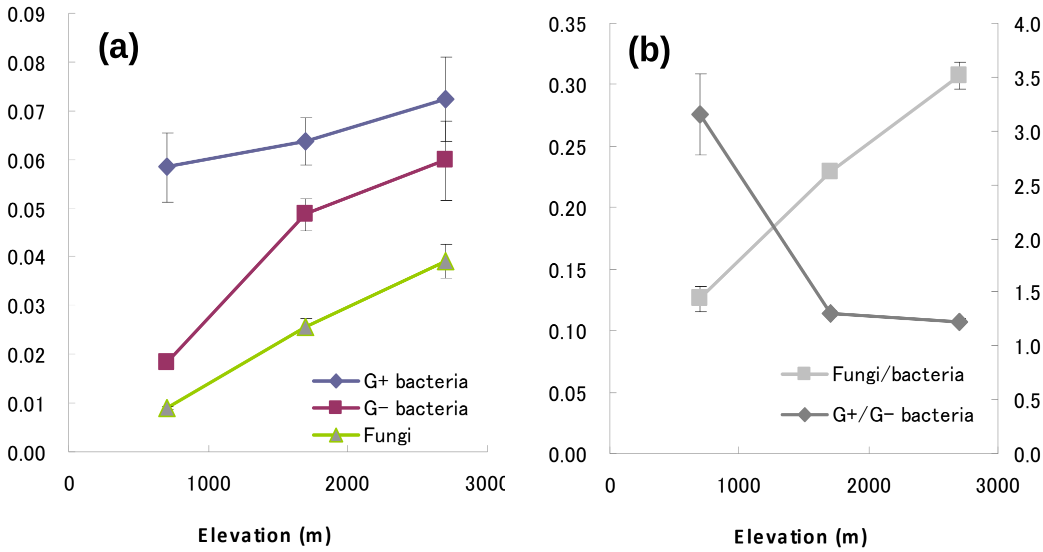


Figure 2. (a) PLFA concentrations of selected broad taxonomic group with elevation (nmol g^{-1}) in sedimentary soils (0-5cm). (b) Ratio of fungi to bacteria (left axis) and gram-positive to gram-negative (right axis) based on (a).

Forest ecosystems at the 700-m elevation are characterized by high rates in plant detritus input (litterfall), rapid decomposition of the detritus in O-horizon, and severe reduction in microbial substrate in mineral horizons due to physical/chemical protection of organic matter by abundant, reactive mineral phases. Our previous work showed that the 700-m soils have the specific surface area of 40-100 m^2 g^{-1} with clay mineralogy of kaolinite and gibbsite at sedimentary site and that of goethite and hematite at ultrabasic site. These mineral phases have significantly greater capacity to adsorb organic substrate and anionic nutrients such as phosphate than upper-elevation soils. Thus microbes in lowland forest soils likely experience rapid changes in physical environment (temperature/moisture) and stochastic pulses of substrate and nutrient input (as litterfall and root death) that are quickly consumed and/or stabilized by soil minerals into unavailable forms. Bacteria appear to be more adapted to these conditions than fungi as the former is capable of rapid growth and dormancy when facing stress (e.g., limitation in substrate and nutrients, draught). Compared to G- bacteria, G+ bacteria hold thicker cellwall, are generally more capable of degrading complex organic substrate and tolerating to stress, and may be more effective in the attachment to soil mineral surfaces. These traits may account for the greater G+ abundance in the 700-m soils.

Depth gradient

Soil samples with depth at each soil pit provide another geochemical gradient in which soil mineralogical characteristics and substrate quality (amounts and chemistry of detritus organic matter) progressively change while macro climate remains constant. With increasing soil depth from surface, the G+:G- ratio among bacteria also increased with depth (Fig. 3a). The ratios for the ultrabasic soils at >60 cm were very high as G+ bacteria was detectable (0.004 nmol g^{-1}) while G- was not detected at all. The fungi+actinomycetes to bacteria ratio showed the lowest values at 10-30 cm and then increased down to 80-100 cm in both sedimentary and ultrabasic soils (Fig. 3b). In a given forest, soils at deeper horizons contain smaller amounts of substrate that are increasingly more stabilized by soil mineral particles and are sparsely located in soil matrix. Fungi and actinomycetes who can extend filaments/hyphae at great extents may thus be more successful utilizing the substrate at depth. Furthermore, fungi (and, to a less extent, G+ bacteria) are capable of producing wider ranges of enzymes to degrade more recalcitrant substrate, which may also account for their relative abundance at depth.

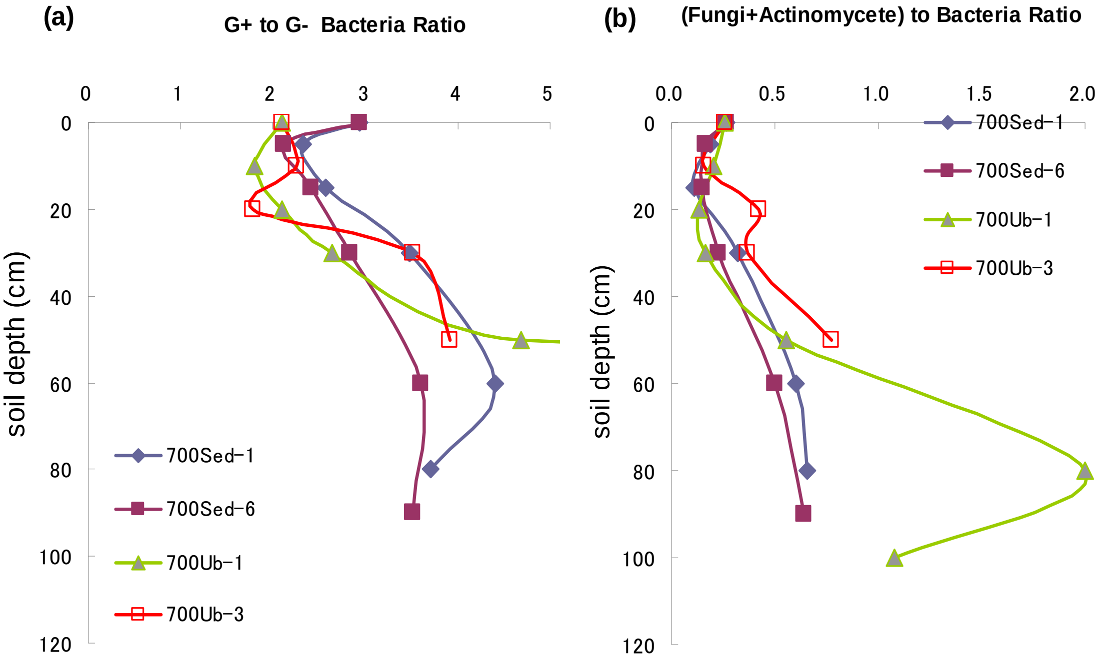


Figure 3. The ratio of gram-positive to gram-negative bacteria (a) and that of fungi plus actinomycete to bacteria (b) along soil profile depth gradients at two 700-m sedimentary soils and two 700-m ultrabasic soils.

Implication for logging impacts

Above results were generally consistent with our hypothesis – the shifts in microbial community composition appeared to be controlled by climate and soil geochemical factors, specifically by temperature, substrate abundance and its interaction with soil mineral particles in the studied ecosystems. Furthermore, we found predictable shifts in microbial community composition corresponding to the changes in climatic and geochemical factors that affect availability of substrate and nutrient. In the environment where organic resources are limited and mineral protection of organic substrate and nutrients is strong, community shifted towards bacterial dominance (particularly G+ ones) over fungi and actinomycetes. Similar shifts in microbial community has been observed along soil depth gradient (down to 2 meter) in Californian grasslands (Fierer *et al.*, 2003).

Then how would soil microbial community respond to the logging of tropical forests? Logging likely causes initial increase and then long-term decline in organic resources due to reduced litter inputs, surface soil erosion, and enhanced decomposition due to disturbance. The prolonged decline in organic resources and enhanced fluctuation in temperature/moisture regime likely result in the community shift towards bacterial dominance. While microbial community hasn’t been characterized with respect to logging intensity, two lines of observations in microbially-driven processes suggest significant shifts in microbial community associated with logging activity.

We compared indicators of soil N and P availability between severely-logged site (Tunkulap) and reduced-logging site (Deramakot). Readily-available ammonium and nitrate in surface soils were roughly two-fold higher at Deramakot than Tunkulap when comparing both on skit trails and under forest canopy (Table 1). Similarly, acid phosphotase activity, microbe- and root-derived enzyme that mineralizes organic phosphorus, was about two-fold greater under reduced-logging sites: 4.04 ± 0.71 in Daramakot and 2.24±0.26 μmol hr^{-1} g^{-1} soil in Tunkulap. These results suggest that severe logging likely resulted in a reduction in nitrogen and phosphorus availability in soil, which was presumably accompanied by shifts in soil microbial community.

Table 1. Comparison of soil total C, N, C:N ratio, and readily-available ammonium and nitrate between skit trail and adjacent forested area. Soil samples (0-10cm deep) were taken from 20-meter long parallel transects along each skit trail (A to E).

Location	TOC	Total N	C:N	KCl−extr NH4+		KCl−extr NO3−	
	%	%		μg/g soil	mgN/gN	μg/g soil	mgN/gN
Deramakot							
Skit trail A	1.27	0.092	13.8	8.1	8.8	5.4	5.8
Forested A	1.99	0.122	16.4	15.0	12.3	5.4	4.4
Skit trail B	1.46	0.108	13.5	12.7	11.8	5.8	5.4
Forested B	2.29	0.161	14.2	9.4	5.8	4.3	2.7
Skit trail C	1.81	0.131	13.9	10.3	7.9	6.0	4.6
Forested C	2.05	0.141	14.5	9.0	6.3	4.7	3.3
Tunkup							
Skit trail D	1.28	0.096	13.3	4.9	5.1	3.4	3.6
Forested D	2.77	0.190	14.6	7.4	3.9	2.9	1.5
Skit trail E	0.98	0.085	11.6	2.5	3.0	-1.6	-1.9
Forested E	2.29	0.162	14.1	4.1	2.5	1.1	0.7

Three samples from each transect were mixed for the chemical analysis.
The inorganic N was extracted by 1.5M potassium chloride solution followed by paper filtration.

To understand logging effects on long-term productivity of tropical forests, it is important to establish direct linkage between microbial community and critical ecosystem processes such as mineralization of N and P. Further investigations on the direct controls on microbial community composition as well as the role of different microbial groups on ecosystem processes would help to substantiate the role of soil microbes in ecosystem functioning and maintenance of biodiversity in tropical forests.

References

Baileya, V.L., Peacockb, A.D., Smithc, J.L., Bolton Jr., H. (2002) Relationships between soil microbial biomass determined by chloroform fumigation–extraction, substrate-induced respiration, and phospholipid fatty acid analysis. Soil Biol Biochem **34**: 1385-1389

Balser, T.C., Tresederb, K.K., Ekenlera, M. (2005) Using lipid analysis and hyphal length to quantify AM and saprotrophic fungal abundance along a soil chronosequence. Soil Biol Biochem **37**: 601-604

Birkeland, P.W. (1999) Soils and Geomorphology, 3rd ed., Oxford Univ. Press, New York. 430. pp

Chapin III, F.S., Matson, P.A., Mooney, H.A. (2002) Principles of terrestrial ecosystem ecology. Springer-Verlag

Fierer, N., Schimel, J.P., Holden, P.A. (2003) Variations in microbial community composition through two soil depth profiles. Soil Biol Biochem **35**: 167-176

Kitayama, K., Aiba, S. (2002) Ecosystem structure and productivity of tropical rainforests along altitudinal gradients under contrasting soil P pools on Mount Kinabalu, Borneo. Jap J Ecol. **90**: 37-51

The Influence of Selective Logging to Decomposition of Leaf Litter in Tropical Forest

Motohiro Hasegawa[1], Masamichi T. Ito[2], Tatsuya Kawaguchi[2]
[1] *Kiso Experimental Station, Forestry and Forest Products Research Institute*
[2] *Yokohama National University*

Introduction

Lavelle (1997) suggested that the role of soil fauna in ecosystems could be divided into three groups, i.e. ecosystem engineer, litter transformer and microbial food web. Litter transformer and ecosystem engineer enhanced litter decomposition processes through their feeding activities (Yamashita and Takeda 1998) and thus have an important ecosystem function. Forest management may affect biological assemblage of decomposers, which may feed back to decomposition rate. However, the influence of forest management on their function in tropical forests has not fully been reported. In our study, the influence of selective logging on the decomposition of leaf litter in tropical forest was investigated and discussed in relation to soil animal activity.

It has been widely recognized that forest disturbance can affect nutrient cycling (Siira-Pietikäinen et al. 2001; Palviainen et al. 2004) and soil biota (Seastedt and Crossley 1981; Holloway et al. 1992; Siira-Pietikäinen et al. 2003; Negrete-Yankelevich et al. 2007).

Selective harvesting or setting logging roads/skid trails in tropical forest might influence the decomposition processes, because they cause the changes of plant community (Burghouts et al. 1994) and climatic conditions (Siira-Pietikäinen et al. 2001), which lead to the changes of soil microbial or faunal components (Davies et al. 1999). Changes in the relative abundance of some groups of soil and litter invertebrates due to selective logging have been reported (Eggleton et al. 1995; Lima et al. 2000).

Recent studies have demonstrated that the soil fauna (mainly soil microarthropods) have a more profound effect on decay rate in wet tropical forests than in dry tropical, temperate and sub-alpine forests (Heneghan et al. 1998; González and Seastedt 2000). Soil macro-fauna (ex. termite, Isopoda and millipedes) contribute to the disappearance of leaf litter by their feeding activities, and their activity results in the loss of leaf area during decomposition. In contrast, during the early stage of leaf-litter decomposition by microorganisms, leaf area does not significantly decrease but the weight of the litter decreases. Thus, it may be possible to evaluate the contribution of soil fauna (especially termites) by measuring the loss of leaf area in comparison to the loss of weight in the initial stage of litter decomposition.

The objective of the study is to investigate the influence of selective logging and skid trail to the decomposition process of tropical tree leaves, and to evaluate the contribution of soil fauna through the analysis of leaf-area loss during decomposition.

Material and Methods

Our study was conducted in the Deramakot Forest Commercial Forest Reserve, Sabah, Malaysia. The details of the forest are shown in Chapters 2 and 3. We set two quadrates (2 X 2m) at the conventionally-logging forest (Tangkulap), the reduced-impact logging forest and the primary forest in Deramakot forest reserve. At each forest, one quadrate was placed at the edge of a skid trail (the width of the trail was 2.5 to 4m), and one quadrate was placed inside the forest (20m from the edge of the trail).

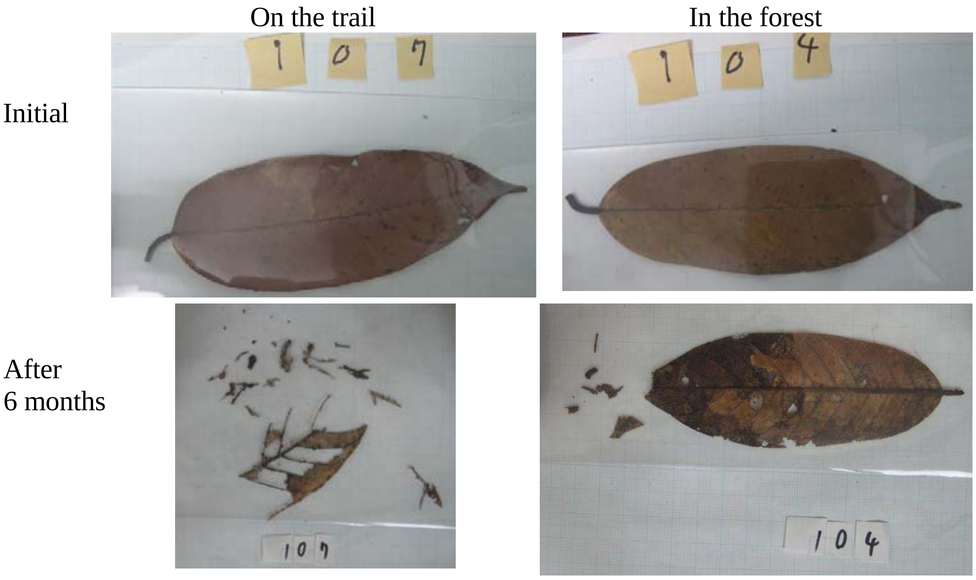


Fig.1 Example of digital photo for leaf area estimation in *Shorea macroptera*

Fresh fallen leaves of *Shorea macroptera* and *Macaranga* sp. were collected from one tree of each species in March 2006. The litter was air-dried and the weight and area of each leaf were measured before placing them into litterbags. The leaf area was analyzed by Image J v. 1.37 (Rasband 2007) using digital image of the leaf. From the 2nd to the 4th March, 2006, we set 10 litterbags of *Shorea macroptera* and *Macaranga* sp. at each quadrate. Litterbags (20 x 20cm) were made of nylon net with a diamond shape mesh (long diagonal line: 4mm, short diagonal line: 2mm). Each litterbag included one piece of leaf for each tree species. On the 30th September, 2006, we collected all of the litterbags. The leaves recovered from the litterbags were measured for wet and air-dried weight as well as leaf area (cf. Fig. 1).

Weight and area remaining were analyzed using two-way analysis of variance (ANOVA) using SYSTAT10.2 (SYSTAT Software Inc., Richmond). The factors were forest type [primary forest (PR), reduced-impact logged forest (RIL) and conventionally-logged forest (CV)] and quadrate placement on the trail (out) and in the forest (in). Data were arcsine transformed before the analysis.

Results

After the 6-month incubation in the field, the percentage of remaining weight relative to the initial weight for *Shorea macroptera* leaf litter ranged from about 16 to 39 %. The percentage of the litter weight in the primary forest was significantly larger than in conventional logging forest and RIL forest (Fig. 2, Table 1). Percentage of remaining weight for Shorea litter was significantly higher within forest than on trail (Fig. 2, Table 1). Significant interaction of two factors was not found (Table1). Percentage of remaining area relative to the initial area for Shorea litter ranged from 33 to 98 %, and it was significantly larger in the primary forest than in the conventionally-logging forest (Fig. 2, Table 1). Percentage of remaining area for Shorea litter was significantly larger within forest than on trail (Fig. 2, Table 1). Significant interaction of two factors was found;

RIL and CV on the trails showed lower percentage of leaf area compared to the other sites (Table1).

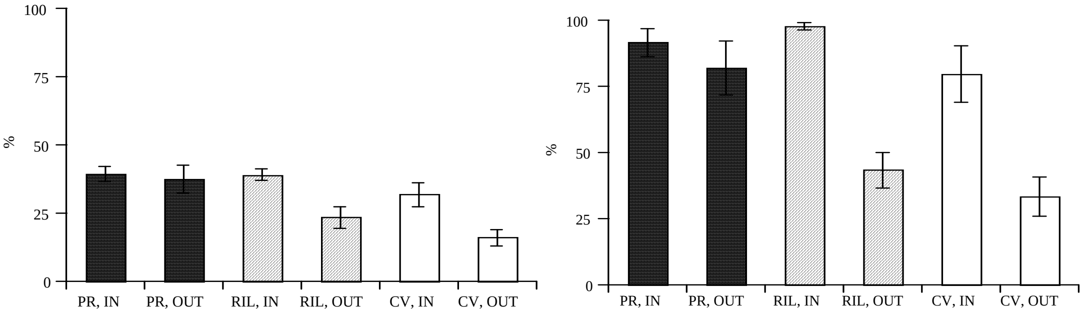


Fig.2 The percentage of remaining weight (left) and area (right) for *Shorea macroptera* leaf litter
PR: Primary forest, RIL: Reduced-impact forest, CV: conventionally-logged forest, IN: within forest, OUT: on the trail. Bars indicate standard errors.

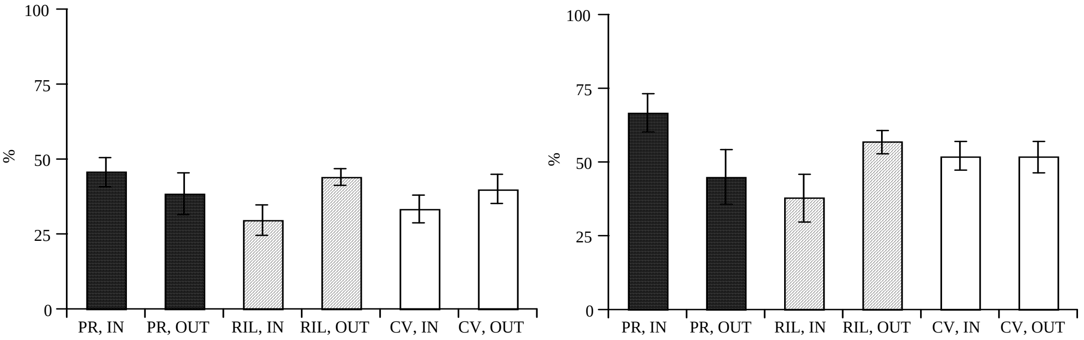


Fig.3 The percentage of remaining weight (left) and area (right) for *Macaranga* sp. leaf litter
Legend as in Fig.2.

Table 1 Results of two-way ANOVA for the effects of forest type and quadrate placement on the weight remaining and leaf area remaining of *Shorea macroptera* litter.

		Shorea macroptera Weight remaining	Area remaining
Forest type	*F*	7.65	9.45
	P	0.001	<0.001
Placement	*F*	13.11	41.18
	P	0.001	<0.001
Forest type X Placement interaction	*F*	2.14	6.76
	P	0.100	0.002

Table 2 Results of two-way ANOVA for the effects of forest type and quadrate placement on the weight remaining and leaf area remaining of *Macaranga* sp. litter.

		Macaranga sp. Weight remaining	Area remaining
Forest type	*F*	0.83	1.20
	P	0.444	0.310
Placement	*F*	1.18	0.12
	P	0.282	0.732
Forest type X Placement interaction	*F*	2.14	4.78
	P	0.128	0.012

The percentage of remaining weight for Macaranga sp. ranged from 30 to 46 %(Fig. 3). No significant difference was found in the weight remaining of two factors and their interaction (Table2). The percentage of remaining area for Macaranga sp. was from 38 to 67 % (Fig. 3). Significant difference in the two factors was not found. However, significant interaction of the two factors was found; the litter placed within the PR forest showed a higher percentage of leaf area than that within the RIL forest (Table2).

Discussion

The percentage of remaining weight after 6months ranged from 16 to 46%. These values are comparable to the previous studies of leaf litter decomposition in tropical forests (Anderson et al. 1983; Yamashita and Takeda 1998; Sundarapandian and Swamy 1999). We expected that the litter of the pioneer species *Macaranga* sp. would decompose faster than that of *Shorea macroptera* because of the physical and chemical characters of *Macaranga* litter (softer texture and higher nutrient concentration). However, the decomposition rate was approximately the same in this study.

On the trail, soil-water content is often lower than in the forest, which likely limits the activity of soil microorganisms. However, the weight loss of *Shorea* leaves was greater on the trail of RIL and CV. The greater weight loss on the trail of RIL and CV was accompanied by the greater rate of the disappearance of leaf area. Activities of soil macrofauna in the litter decomposition involve the loss of weight and area of litter. Therefore, the difference in remaining leaf area may be attributable to macro-faunal feeding activity. The difference in the loss of weight and area between within-forest and trail was more prominent in *Shorea* leaves than in *Macaranga* leaves. Animal feeding habit might have contributed to the different pattern of decomposition between tree species.

In tropical forests, termites are an important faunal component for litter decomposition (Abe and Matsumoto 1979; Petersen and Luxton 1982). Yamashita and Takeda (1998) suggested that the soil animals especially termites accelerated leaf litter decomposition by their feeding in Malaysian tropical forests. Lima et al. (2000) suggested that disturbances such as logging which reduces the abundance of some species of termites could reduce the rate of litter breakdown inside a forest.

In our study area, however, the density of termites was not so high, compared with other sites in tropical area (Abe and Matsumoto 1979; Eggleton et al. 1999; Hasegawa et al. 2006). Burghouts et al. (1992) also suggested that the termite density in Danum Valley in Sabah was smaller than that in the other sites in Asia and Africa. In Borneo, Eggleton et al. (1999) suggested that selective logging appeared to have relatively little effect on termite assemblages, although soil-feeding termites may be moderately affected by this level of' disturbance. In the same study area as our current study, Hasegawa et al. (2006) suggested that the density of termites in the PR site was higher than that in the CV. Thus, the contribution of termites to the difference in decomposition between the PR and CV sites might be less important. However, termite distribution is generally heterogeneous, and they can bring litter to their nests, that might have not been located in our sampling zone. Thus, we might have missed the activity of termites in the CV site. In addition, we did not identify the species of termites. Some important groups of termites (ex. *Macrotermes*) may be included in the CV sites. In contrast, Hasegawa et al. (2006) suggested that isopoda and diplopoda density was higher in the CV sites. Tsukamoto and Sabang (2005) suggested that earthworm and isopods increased in *Acacia* plantation than in mixed Dipterocarps forest. Hassall et al. (2006) also suggested that the relative abundance of individual species of Isopoda was highest in the most disturbed environment. Further ecological study of termites and Isopoda are needed.

In conclusion, conventional logging and the creation of skid trails in this study area might accelerate the decomposition process. Greater litter fragmentation may enhance surface runoff of fragmented litter upon rainfall events. The enhanced runoff may aggravate the condition for the colonization of other soil biota and tree seedling, and may lead to a successive loss of biodiversity in that area (Tsukamoto and Sabang 2005; Ruan et al. 2005).

References

Abe T, and Matsumoto T (1979) Studies on the distribution and ecological role of termites in a lowland rain forest of west Malaysia (3) Distribution and abundance of termites in Pasoh forest reserve. Jap J Ecol **29**:337-351

Anderson J M, Proctor J, Vallack H W (1983) Ecolological studies in four contrasting lowland rain forests in Gunung Mulu national Park, Sarawak. Journal of Ecology **71**:503-527

Burghouts T, Ernsting G, Korthalis G, De Vries T (1992) Litterfall, leaf litter decomposition and litter invertebrates in primary and selectively logged dipterocarp forest in Sabah, Malaysia. Phil. Trans. R. Soc. Lond. B **335**:407-416

Burghouts T B A, Campbell E J F, Kolderman P. J. (1994) Effects of Tree Species Heterogeneity on Leaf Fall in Primary and Logged Dipterocarp Forest in the Ulu Segama Forest Reserve, Sabah, Malaysia. J Trop Ecol **10**:1-26

Davies R G, Eggleton P, Dibog L, Lawton H, Bignell D E, Brauman A, Hartmann C, Nunes L, Holt J, Rouland C (1999) Successional response of a tropical forest termite assemblage to experimental habitat perturbation. J Appl Ecol **36**:946-962

Eggleton P, Bignell D E, Sands W A, Waite B, Wood T G, Lawton J H (1995) The Species Richness of Termites (Isoptera) Under Differing Levels of Forest Disturbance in the Mbalmayo Forest Reserve, Southern Cameroon. J Trop Ecol **11**:85-98

Eggleton P, Homathevi R, Jones D T, MacDonald J A, Jeeva D, Bignell D E, Davies R G, Maryati M (1999) Termite assamblages, forest disturbance and greenhouse gas fluxes in Sabah, East Malaysia. Phil Trans R Soc Lond B **354**:1791-1802

González G, Seastedt T R (2000) Comparison of the abundance and composition of litter fauna in tropical and sub-alpine forests. Pedobiologia **44**:545-555

Hasegawa M, Ito M T, Kitayama K, Seino T, Chung A Y C (2006) Logging effects on soil macrofauna in rain forests of Deramakot Forest Reserve, Sabah, Malaysia. The second Workshops on synergy between carbon management and biodiversity conservation in tropical rain forests, Workshop proceedings:53-60

Hassall M, Jones D, Taiti S, Latipi Z, Sutton S L, Mohammed M (2006) Biodiversity and abundance of terrestrial isopods along a gradient of disturbance in Sabah, East Malaysia. Eur J Soil Biol 42 (2006) **42,** supplement:197–207

Heneghan L, Coleman D C, Zou X, Crossley Jr. D A, Haines B L (1998) Soil microarthopod community structure and litter decomposition dynamics: A study of tropical and temperate sites. Appl Soil Ecol **9**:33-38

Holloway J D, Kirk-Spriggs A H, Khen C V (1992) The Response of Some Rain Forest Insect Groups to Logging and Conversion to Plantation. Phil Trans R Soc Lond B **335**:425-436

Lavelle, P (1997) Faunal activities and soil processes: Adaptive strategies that determine ecosystem function. Advances in Ecological Research **27**:91-132.

Lima A P, Cordeiro-Duarte A C, Luizao F J, Higuchi N (2000) Effect of selective logging intensity on two termite species of the genus Syntermes in Central Amazonia. For Ecol Manag **137**:151-154

Negrete-Yankelevich S, Fragoso C, Newton A C, Heal O W (2007) Successional changes in soil, litter and macroinvertebrate parameters following selective logging in a Mexican Cloud Forest. Appl Soil Ecol **35**:340-355

Palviainen M, Finer L, Kurka A M, Mannerkoski H, Piirainen S, Starr M (2004) Decomposition and nutrient release from logging residues after clear-cutting of mixed boreal forest. Plant and Soil **263**:53-67

Petersen H, Luxton M (1982) A comparative analysis of soil fauna populations and their role in decomposition processes. Oikos **39**:288-388

Rasband W S (2007) ImageJ, V. 1.37 U. S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/.

Ruan, H., Y. Li, and X. Zou. 2005. Soil communities and plant litter decomposition as influenced by forest debris: Variation across tropical riparian and upland sites. Pedobiologia **49** 529—538.

Seastedt T R, Crossley Jr D A (1981) Microarthropod response following cable logging and clear-cuting in the Southern Apparachians. Ecology **62**:126-135

Siira-Pietikäinen A, Haimi J, Siitonen J (2003) Short-term responses of soil macroarthropod community to clear felling and alternative forest regeneration methods. For Ecol Manag **172**:339–353

Siira-Pietikainen A, Pietikainen J, Fritze H, Haimi J. (2001) Short term responses of soil decomposer communities to forest management: clear felling versus alternative forest harvesting methods. Can J For Res **31**:88-99

Sundarapandian S M, Swamy P S (1999) Litter production and leaf-litter decomposition of selected tree species in tropical forests at Kodayar in the Western Ghats, India. For Ecol Manag **123** 231-244

Tsukamoto J, Sabang J (2005) Soil macro-fauna in an *Acacia mangium* plantationin comparison to that in a primary mixed dipterocarp forest in the lowlands of Sarawak, Malaysia. Pedobiologia **19**:69-80

Yamashita T, Takeda H (1998) Decomposition and nutrient dynamics of leaf litter in litter bags of two mesh sizes set in two dipterocarp forest sites in Penisular Malaysia. Pedobiologia **42**:11-21