

Relationships between physical and chemical characteristics of dipterocarp seeds

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Abstract

The physical and chemical seed characteristics of 11 dipterocarp species (four *Dipterocarpus*, two *Dryobalanops* and five *Shorea* species) were quantified to examine variations among species. We measured dry weight, pericarp thickness and concentrations of nitrogen, total phenol, condensed tannin, lignin, lipid, starch and total sugar. Although there was low intraspecific variation among parent trees, each species had unique characteristics. We found significant correlations between chemical defensive compounds (i.e. lignin and total phenol) and nitrogen concentration. However, these relationships were no longer significant when an outlier was removed. Fruiting frequency was not significantly correlated with any chemical compound. Seeds of *Shorea* species had a higher concentration of lipid than seeds of *Dipterocarpus*. The implications of variation in the physical and chemical characteristics of dipterocarp seeds are discussed.

Keywords: defence, *Dipterocarpaceae*, general flowering, nitrogen concentration, resource allocation, Sarawak, total phenol

Introduction

For many plants, the seed represents a critical stage in regeneration, as seeds experience the highest mortality during the life cycle, primarily as a result of predation (Crawley, 1992; Tanaka, 1995). One of the most effective defence mechanisms employed to protect seeds is the production of chemical compounds (Smallwood and Peters, 1986; Aganga and Mosase, 2001). Physical characteristics, such as a thick or a hard seed coat, can also serve as defences (Blate *et al.*, 1998; Kollmann *et al.*, 1998), and seed size may affect predator preferences (Blate *et al.*, 1998;

Grubb and Burslem, 1998; Grubb *et al.*, 1998). On the other hand, for their offspring, plants must also allocate nutrient resources (e.g. lipid or starch), which influence predator preferences (Laska, 2001). Some vertebrates prefer seeds that have a higher protein concentration (Wendeln *et al.*, 2000). This suggests that preferred seeds may possess defensive systems. In fact, in an Australian tropical rainforest, Grubb *et al.* (1998) found that the better-defended seeds had a notably higher concentration of nitrogen. However, little information is available on seed characteristics in South-East Asian tropical rainforests (Grubb and Burslem, 1998; Numata *et al.*, 1999), and nothing has been published about the relationship between chemical defensive substances and nitrogen concentration.

Masting, i.e. the intermittent production of large seed crops at the population level, may also help to protect seeds by satiating seed predators (Kelly, 1994). Many reports have examined and tested this satiation hypothesis (e.g. Kelly *et al.*, 2000; Shibata *et al.*, 2002). Lowland mixed dipterocarp forests in South-East Asia experience a unique phenomenon, termed general flowering (GF), during which masting at intervals of 2–10 years occurs not only at the population level, but also at the guild and community levels (Sakai, 2002). In these forests, plants may need to invest less energy in accumulating defence compounds because seed predators can become saturated by the synchronous production of seeds. Numata *et al.* (1999) tested the hypothesis that *Shorea* species with a lower total phenolic concentration produced seeds less frequently. However, they examined only one genus, *Shorea*, and only one defensive substance (total phenolic concentration); to date, no studies have considered other dipterocarp genera or other compounds used in defence or storage.

Therefore, the aims of this study were: (1) to quantify the physical characteristics and concentrations of chemical compounds in the seeds of 11 species (in three genera) of *Dipterocarpaceae* – trees of this family dominate the emergent layer in lowland mixed

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dipterocarp forests and are the principal participants in GF (Ashton, 1982; Sakai *et al.*, 1999); and (2) to test the following specific hypotheses: (a) seeds protected by a thick pericarp or by chemical compounds (total phenol and lignin) have higher nitrogen concentrations; and (b) seeds with a shorter fruiting period have higher concentrations of defence or storage substances. Finally, we examined seed characteristics synthetically using principal components analysis.

Materials and methods

Study site and plants

The study was conducted in Lambir Hills National Park, Sarawak, Malaysia (4°20'N, 113°50'E; altitude 150–250 m). The park is covered mostly with primary lowland mixed dipterocarp forest. The flowering and fruiting phenologies of more than 300 species have been observed since 1992, using a canopy observation system (Inoue *et al.*, 1995). General flowering (GF) occurred from August 2001 to March 2002 in Lambir Hills National Park, and 16 species out of 36 observed dipterocarps participated in this event (Sakai, unpublished data). GF has been observed four times since the beginning of the phenology census in our study plot, and the scale of the GF event in 2001–02 (26.2% of observed trees flowered) was larger than those in 1997 (19.5%) and 1998 (21.1%), but smaller than the GF observed in 1996 (29.7%) (Kato *et al.*, 2000; Sakai, unpublished data).

Two long-term study plots have been established in the park: the 8-ha Canopy Biology Plot (CBP) was established in 1992, and the 4-ha Crane Plot (CP) was established in 2000. We sampled non-damaged and mature dipterocarp fruits of 11 species in three genera (*Dipterocarpus*, *Dryobalanops* and *Shorea*) in and

around the CBP and the CP from January to March 2002, when mature fruits were dispersed (Table 1). When possible, sampling was conducted in the canopy; otherwise, we collected fruits on the ground shortly after seedfall. Fruits were frozen at –20°C as soon as possible after collection.

All of the studied dipterocarps produce one-seeded and exalbuminous seeds. The seeds are surrounded by the pericarp and sepals and do not become dormant. In this paper, we considered embryos to be seeds. Since most dipterocarp seed predators use neither the pericarp nor the sepals, but only the embryos (M. Nakagawa, personal observation), and the testa is very thin, we analysed only the embryos.

Physical characteristics and chemical analysis

Since some species produced a small quantity of sound and mature seeds, while in other species, only a single tree fruited, we quantified 3–15 seeds from 1–5 parent trees (Table 1). If seeds were partly infested by insects or damaged by fungi, they were not included in the analysis. Pericarp thickness was measured with vernier calipers. The seeds were freeze-dried (EYELA FDU-540, Tokyo, Japan) for 2 d, weighed, and then ground to a fine powder, one at a time, in a mortar.

The concentrations of total phenolics and condensed tannin in the seeds were quantified by extraction with 50% methanol and determined by the Folin–Ciocalteu and proanthocyanidin methods, respectively (Julkunen-Tiitto, 1985; Waterman and Mole, 1994). The standards for the assays were tannic acid for total phenolics and cyanidin chloride for condensed tannin. Lignin was extracted with 80% ethanol at 40°C for at least 16 h; lignin concentration was then determined by the acetyl bromide

Table 1. List of dipterocarp species analysed, with plant species abbreviation code, the number of seeds (*n*) and number of parent trees. Mean dry weight ± SD, pericarp thickness, and number of sepals (wing) are also shown

Species ^a	Code	<i>n</i>	Number of parent trees	Dry weight of a seed (g)	Pericarp thickness (mm)	Wing (long + short)
<i>Dipterocarpus globosus</i>	DGL	3	1	3.79 ± 0.54	5.26 ± 0.80	2 + 3
<i>D. pachyphyllus</i>	DPC	3	2	0.28 ± 0.26	1.00 ± 0.25	2 + 3
<i>D. palembanicus</i>	DPL	9	2	0.88 ± 0.05	2.23 ± 0.87	2 + 3
<i>D. tempehes</i>	DTE	9	3	2.33 ± 0.88	3.01 ± 0.52	0
<i>Dryobalanops aromatica</i>	DAR	15	5	1.85 ± 0.71	1.09 ± 0.19	5
<i>Dr. lanceolata</i>	DLA	9	3	2.04 ± 0.52	1.17 ± 0.14	5
<i>Shorea beccariana</i>	SBE	12	4	0.32 ± 0.09	2.18 ± 0.48	3 + 2
<i>S. laxa</i>	SLA	3	1	7.61 ± 0.95	1.58 ± 0.25	0
<i>S. parvifolia</i>	SPA	3	1	0.33 ± 0.12	0.24 ± 0.03	3 + 2
<i>S. pilosa</i>	SPI	3	1	2.88 ± 1.34	1.91 ± 0.22	3 + 2
<i>S. smithiana</i>	SSM	3	1	1.11 ± 0.13	0.89 ± 0.09	3 + 2

^a Nomenclature follows Ashton (1982).

procedure, as modified by Iiyama and Wallis (1988, 1990) and Hatfield *et al.* (1999). Lipid concentration was analysed by the Soxhlet extraction method (Allen, 1974). Total sugars were extracted at 90°C for 16 h in 80% ethanol, then quantified using the phenol-sulphuric acid method (Dubois *et al.*, 1956; Ashwell, 1966). The residue of the ethanol extract for total sugar was saved for starch analysis, enzymatically reduced to glucose, and quantified using the phenol-sulphuric acid method (Hewitt and Marrush, 1986; Newell *et al.*, 2002). The fractional transmittance was read in a UV/VIS spectrophotometer (SHIMADZU UV-2500PC, Kyoto, Japan), and the nitrogen concentration was measured using a CHN Corder (Yanako MT-3, Kyoto, Japan).

Fruiting frequency

The fruiting phenologies of 10 of the 11 study species (no data for *Shorea laxa*) have been monitored in the study plot since 1993, as part of a phenology census (Sakai *et al.*, 1999), which includes 3–11 individuals of the 10 species. We defined fruiting frequency as the number of fruiting years between 1993 and 2001 when at least one tree produced fruit.

Statistical analyses

We used the Kruskal–Wallis test to analyse variation of each seed characteristic and to detect significant differences among all species. The three specific hypotheses were evaluated by Pearson correlation coefficients. A *t*-test was used to examine inter-genus differences between *Dipterocarpus* and *Shorea*. Principal components analysis (PCA) was performed on chemical and physical characteristics of dipterocarp seeds, to examine variations among species. We used

the statistical package STATISTICA 5.1 (StatSoft, 1995) for all analyses.

Results

Chemical characteristics of dipterocarp seeds

All chemical compounds found in seeds differed significantly among species (Table 2). The highest concentration of condensed tannin was measured in *Dryobalanops lanceolata*. *Dipterocarpus palembanicus* had the highest total phenol, lignin and nitrogen concentrations. *Shorea beccariana* and *S. laxa* seeds contained the highest concentrations of total sugar and starch, respectively, among the species studied, while *S. parvifolia* had the highest lipid concentration (Table 2). Four species (*D. globosus*, *D. pachyphyllus*, *D. tempehes* and *S. laxa*) did not contain condensed tannin.

Correlation between concentration of nitrogen and degrees of defence

We found a significant positive correlation between nitrogen concentration and lignin or total phenol concentrations, whereas nitrogen concentration was not significantly correlated with pericarp thickness (Fig. 1). However, when the values for an outlier, *D. palembanicus*, were removed, the differences were no longer significant. There was no significant separation of species by genus in terms of nitrogen, total phenol and lignin concentrations, whereas *Dipterocarpus* species, with the exception of *D. palembanicus*, showed a tendency to have a lower concentration of total phenols than the *Shorea* species; the two *Dryobalanops* species were intermediate (Fig. 1).

Table 2. Mean concentrations of chemical compounds in mature dipterocarp seeds \pm SD [mg (g dried weight)⁻¹]. Values for Kruskal–Wallis test (H and P) are also shown

Species	<i>n</i>	Nitrogen	Total phenol	Condensed tannin	Lignin	Lipid	Starch	Total sugar
<i>D. globosus</i>	3	7.9 \pm 1.4	6.3 \pm 1.8	0.0 \pm 0.0	11.6 \pm 1.2	64.1 \pm 6.3	746.5 \pm 60.7	42.2 \pm 6.9
<i>D. pachyphyllus</i>	3	9.0 \pm 6.6	5.3 \pm 3.0	0.0 \pm 0.0	15.1 \pm 10.5	84.7 \pm 19.2	530.0 \pm 72.4	294.9 \pm 232.9
<i>D. palembanicus</i>	9	17.9 \pm 5.1	440.0 \pm 57.8	6.2 \pm 3.2	44.1 \pm 10.0	4.5 \pm 10.9	249.6 \pm 139.3	214.7 \pm 60.8
<i>D. tempehes</i>	9	7.3 \pm 1.3	2.7 \pm 2.1	0.0 \pm 0.0	14.4 \pm 3.2	47.9 \pm 7.9	714.1 \pm 85.9	86.8 \pm 28.1
<i>Dr. aromatica</i>	15	9.7 \pm 2.2	12.7 \pm 5.1	3.1 \pm 1.6	16.0 \pm 5.9	406.2 \pm 38.8	281.6 \pm 56.1	57.0 \pm 16.2
<i>Dr. lanceolata</i>	9	7.8 \pm 1.1	63.9 \pm 21.9	8.0 \pm 4.7	27.2 \pm 9.2	155.3 \pm 18.9	464.7 \pm 57.1	61.5 \pm 19.1
<i>S. beccariana</i>	12	7.3 \pm 3.5	228.5 \pm 71.7	1.1 \pm 1.5	26.1 \pm 6.7	180.2 \pm 172.8	132.1 \pm 90.9	399.6 \pm 176.9
<i>S. laxa</i>	3	5.4 \pm 0.7	47.1 \pm 13.6	0.0 \pm 0.0	10.7 \pm 1.0	2.9 \pm 1.4	829.4 \pm 14.9	53.4 \pm 13.2
<i>S. parvifolia</i>	3	7.7 \pm 0.5	60.5 \pm 13.4	1.6 \pm 0.8	24.5 \pm 1.4	499.0 \pm 5.1	202.0 \pm 24.7	61.0 \pm 21.9
<i>S. pilosa</i>	3	2.6 \pm 2.4	74.2 \pm 6.2	2.1 \pm 0.7	17.4 \pm 1.9	491.5 \pm 6.7	90.7 \pm 27.6	85.8 \pm 21.2
<i>S. smithiana</i>	3	7.8 \pm 0.4	100.0 \pm 47.9	0.4 \pm 0.4	16.8 \pm 3.5	373.5 \pm 3.1	197.2 \pm 22.9	56.7 \pm 22.4
H		45.7	64.5	49.5	40.6	54.1	46.4	50.0
P		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

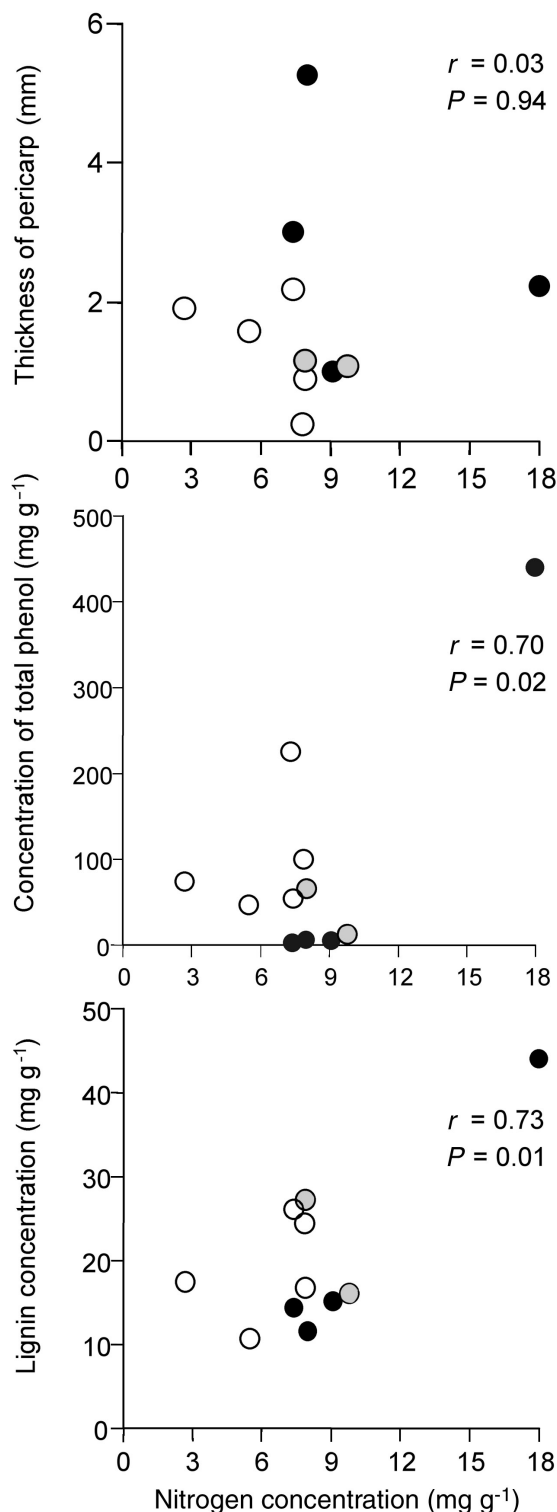


Figure 1. Correlations between nitrogen concentration and pericarp thickness, and concentration of total phenolics and lignin of 11 dipterocarp species. The symbols represent a genus: closed circles, *Dipterocarpus*; shaded circles, *Dryobalanops*; and open circles, *Shorea*.

Correlations between fruiting frequency and chemical compounds

With the exception of starch, all chemical compounds tended to increase in concentration with fruiting frequency, although none of these correlations was significant. Starch concentration was negatively, though not significantly, correlated with fruiting frequency.

Physical and chemical variations among dipterocarps

Axis 1 (PC1) of the principal component ordination of chemical and physical characteristics accounted for 36.1% of the total variation among dipterocarp seeds, and PC2 added another 26.8% (Fig. 2). Large negative contributions to PC1 were made by concentrations of nitrogen, total phenol, condensed tannin and lignin. On the other hand, PC2 was negatively correlated with lipid concentration and positively correlated with starch concentration and pericarp thickness.

PC3, which explained 18.5% of the total variation, was positively correlated with seed weight and negatively correlated with total sugar concentration. *D. palembanicus* had the most outstanding characteristics in terms of extremely high concentrations of lignin, condensed tannin, total phenol and nitrogen (Fig. 2). Although most species were scattered continuously, and we detected no clear clusters among genera or sections, *Dipterocarpus* species tended to have lower PC2 values, suggesting that they had lower and higher concentrations of lipid and starch, respectively (Fig. 2). Seeds of *Dipterocarpus* had significantly lower concentrations of lipids than those of *Shorea* (*t*-test, $P < 0.05$).

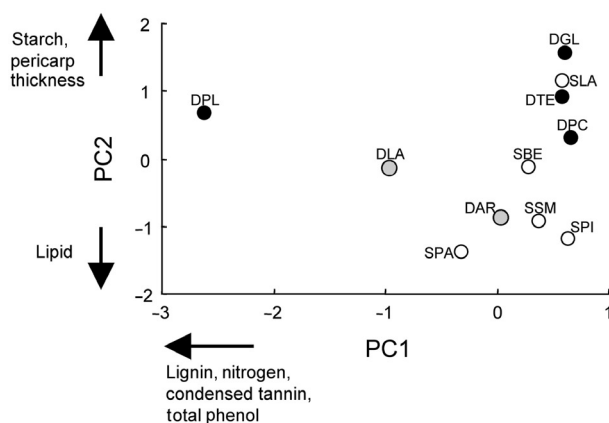


Figure 2. Two-dimensional scatter diagram of principal components analysis (PCA) for seeds of 11 dipterocarp species ordinated on the physical and chemical characteristics. The symbols represent a genus: closed circles, *Dipterocarpus*; shaded circles, *Dryobalanops*; and open circles, *Shorea*. For the species codes, see Table 1.

Discussion

Limitations of chemical analyses

When major storage substances [lipid, protein (converted from nitrogen multiplied by 6.25), starch and total sugar] and a cell wall component (lignin) were summed, the values ranged from c. 560 to c. 970 mg g⁻¹. We did not quantify the concentrations of cellulose, hemicellulose and ash, although these fractions in various seeds have been reported as 1.3–4.7%, 0.7–22.7% and 4.5–32.1% dry weight, respectively (Ofcarcik and Burns, 1971; Short, 1976; Short and Epps, 1976; Esuoso *et al.*, 1998; Wendeln *et al.*, 2000). Although the sum of these three chemicals and values of our results exceed 1000 mg g⁻¹, the comparisons among values found in this study are not affected.

Interspecific variation in chemical compounds

In most cases, we analysed the chemical compounds for only one tree of each species, but for three species, *D. tempehes*, *Dr. aromatica*, and *S. beccariana*, which had the most parent trees in each genus, concentrations of chemical compounds were similar in different trees. Although concentrations varied somewhat among individual trees of the same species, most differences were not significant (Kruskal–Wallis test, $P > 0.05$). Only the concentration of condensed tannin differed significantly among individual *S. beccariana* ($H = 9.97$, $P = 0.019$). Thus, whereas one chemical compound did show significant differences among parent trees, overall, chemical compounds and their compositions in dipterocarp seeds did not differ widely among individual trees within species. Therefore, although for several species we sampled mature seeds from only a single parent tree and the sample size of all species was relatively small, the concentrations of chemical compounds that we measured are representative of the species.

Physical and chemical properties of dipterocarp seeds

Our first hypothesis, regarding nitrogen concentration and physical or chemical defences, was not supported when the outlier, *D. palembanicus*, was excluded. This result suggests that defensive systems in dipterocarp seeds by means of physical characteristics (pericarp thickness) and chemical compounds (total phenol and lignin) may not be well developed, and that there may be another means in place for preventing seed predation. One possible system for avoiding seed predation could be synchronous fruiting among various species, such as GF, in order to satiate seed predators; this hypothesis has been discussed as one of the most likely explanations with respect to the

ultimate impact of GF (Janzen, 1974; Sakai, 2002). However, our second hypothesis was not supported, as neither defensive substances nor storage compounds were correlated with fruiting frequency. Thus, the characteristics of dipterocarp seeds may be regulated not only by protection from seed predators, but also by substitute benefits of seed dispersal or seedling establishment.

Across all species, seed characteristics changed continuously, and we did not find any apparent association between physical and chemical seed properties and genus. However, the composition of chemical compounds in dipterocarp seeds differed significantly among species. In particular, *D. palembanicus* had unique characteristics, with a high concentration of both defensive substances and nitrogen. Ecological study on seeds and seedlings of *D. palembanicus* will permit an understanding of the significance of these unique properties.

Among the three genera studied, *Dipterocarpus* species, with the exception of *D. palembanicus*, had properties that differed somewhat from those of *Dryobalanops* and *Shorea*. A significant difference between *Dipterocarpus* and *Shorea* was detected only in lipid concentrations, in that *Shorea* seeds had a higher lipid concentration than those of *Dipterocarpus*. In this study, while seeds of *Dryobalanops* were excluded from inter-genus examination because of the small sample size, their characteristics were similar, relatively speaking, to those of *Shorea*. A trend emerged, in that *Dipterocarpus* seeds had a lower total phenol concentration and a higher starch concentration, while the other genera had a higher total phenol concentration with a higher lipid concentration. Given the many reports on the preferences of seed predators for lipid-rich seeds (Smith and Follmer, 1972; Smallwood and Peters, 1986), the selection for protection by total phenol in seeds with a higher lipid concentration may have been precipitated by increased predation pressure.

Further studies of the physical and chemical characteristics of other dipterocarp species or genera, as well as other families, are necessary for a comprehensive understanding of the significance of seed content. We also need to investigate seed predation rates by vertebrates, and the survivorship and initial growth of seedlings, to elucidate the ecological implications of variations in seed characteristics.

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