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SPECIES AND GENETIC DIVERSITY OF FAGACEAE IN NORTHERN THAILAND BASED ON ISSR MARKERS

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CHOKCHAICHAMNANKIT, P., CHULALAKSANANUKUL, W., PHENGKLAI, C. & ANAMTHAWAT-JÓNSSON, K. 2008. Species and genetic diversity of Fagaceae in northern Thailand based on ISSR markers. The family Fagaceae in northern Thailand consists mainly of three genera: *Castanopsis*, *Lithocarpus* and *Quercus*, each with extensive diversity at species level. A study area of about 550 km^2 in Doi Saket district, Chiang Mai province was selected where Fagaceae can be found in different habitats and forest types. Samples were collected from 146 randomly selected trees: leaves, fruits (acorns) and flowers for taxonomic identification, and young leaves for molecular analysis. Based on the morphological characters, 30 species were identified from this collection. Twelve species of *Castanopsis* were discovered, with *C. acuminatissima* being the most dominant species especially in hill-evergreen forests. Eleven species of *Quercus* were found essentially at lower altitudes and mainly in deciduous dipterocarp forests, with *Q. kerrii* being the most common. *Lithocarpus* included seven species and the genus was found in all forest types. However, the most common species, *L. harmandianus* was found mainly in hill-evergreen forest with pine. Phenetic relationship among these species was analyzed using inter-simple sequence repeats (ISSR) markers. The results showed clear separation of the genera and of most species, supporting the taxonomic classification. Some *Lithocarpus* samples were found within the *Castanopsis* group. The study reported here is seen as the first step aiming at characterizing diversity of one of the most important tree families in a region where taxonomic diversity is large and mostly undescribed.

Keywords: *Castanopsis*, *Lithocarpus*, *Quercus*, phenetic relationship, molecular diversity

CHOKCHAICHAMNANKIT, P., CHULALAKSANANUKUL, W., PHENGKLAI, C. & ANAMTHAWAT-JÓNSSON, K. 2008. Kepelbagaian spesies dan genetik Fagaceae di utara Thailand berdasarkan penanda ISSR. Famili Fagaceae di utara Thailand terdiri terutamanya daripada tiga genus iaitu *Castanopsis, Lithocarpus* and *Quercus*. Kepelbagaian spesies setiap genus ini sangat luas. Satu kajian dijalankan di sebuah kawasan seluas 550 km^2 di daerah Doi Saket, Chiang Mai. Di sini Fagaceae ditemui di habitat dan jenis hutan yang berbeza-beza. Sampel dikumpul daripada 146 pokok yang dipilih secara rawak. Daun, buah (akorn) dan bunga diambil untuk pengecaman taksonomi sementara daun muda untuk analisis molekul. Berdasarkan ciri morfologi, kami telah mengecam 30 spesies. Dua belas spesies *Castanopsis* ditemui dan antaranya *C. acuminatissima* merupakan spesies paling dominan khususnya di hutan bukit malar hijau. Sebelas spesies *Quercus* ditemui di altitud yang lebih rendah, terutamanya di hutan dipterokarpa daun luruh dan *Q. kerrii* merupakan spesies utama. Terdapat tujuh spesies *Lithocarpus* dan genus ini ditemui di semua jenis hutan. Bagaimanapun, spesies yang paling biasa ditemui ialah *L. harmandianus* yang tumbuh di hutan bukit malar hijau bersama-sama pokok pain. Hubungan fenetik antara semua spesies ini dianalisis menggunakan penanda ISSR. Keputusan menunjukkan pemisahan yang jelas bagi genus dan kebanyakan spesies, sesuai dengan pengelasan taksonominya. Sesetengah sampel *Lithocarpus* didapati dalam kumpulan *Castanopsis.* Kajian ini merupakan usaha pertama untuk mengelaskan kepelbagaian salah satu daripada famili pokok yang paling penting di kawasan yang mempunyai kepelbagaian taksonomi yang luas serta belum diperihalkan.

INTRODUCTION

The beech family (Fagaceae) dominates forests in the temperate, seasonally dry regions of the Northern Hemisphere with a centre of diversity found in tropical South-East Asia, particularly at the generic level (Manos *et al.* 2001). The family includes 7–12 genera and 600–1000 species worldwide (Soepadmo 1972, Scoggan 1978, Mabberley 1997, Chengjiu *et al.* 1999).

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In Thailand this family comprises four genera (Phengklai *et al.* 2006), namely, *Castanopsis* (D. Don) Spach. (chestnut, mostly evergreen, 33 species), *Lithocarpus* Blume (stone oak, mostly evergreen, 56 species), *Quercus* L. (oak, mostly deciduous, 29 species) and *Trigonobalanus* Forman (evergreen, one species). In northern Thailand, the number of Fagaceae species is estimated to be 40–60 (Phengklai *et al.* 2006), or about 4–10% of the total world species diversity in this family. Habitats of Fagaceae are relatively diverse; while being most common in hill-evergreen forests it is also found in dry-deciduous dipterocarp forests at lower altitudes and mixed-deciduous forests with open grassland (Gardner *et al.* 2000). Montane forests in northern Thailand, including Fagaceae-dominating hill-evergreen forests, serve as an important origin of water supply and also of streams that flow into the lowland agricultural regions of the country, including the Chaophraya river basin. Edible nuts of *Castanopsis* (e.g. *C. diversifolia* and *C. acuminatissima*) are very much sought after by the locals. Fagaceae from this region provides generally low-quality timber but makes excellent firewood. It has also been used for growing Shiitake mushrooms.

Species diversity of Fagaceae in Thailand is substantial, as indicated by the initial survey of Fagaceae in Thailand (Smitinand 1980). Over 100 species have been documented in the latest revision of the *Flora of Thailand* (Phengklai *et al.* 2006). Three new species and a new variety have recently been described (Phengklai 2004). In practice, identification of Fagaceae species is known to be difficult due to many reasons including extensive intraspecific morphological variation, ecological adaptation, clinal differentiation, hybridization, gene flow and introgression. These phenomena have been well documented especially in oaks (*Quercus*) of Europe and North America (Schwarz 1964, Scoggan 1978, Dumolin-Lapegue *et al.* 1997, Montalvo *et al.* 1997, Streiff *et al.* 1998, Bellarosa *et al.* 2005, Gonzalez-Rodriguez *et al.* 2005). The level of genetic diversity within the genus *Quercus* in Eastern Asia also appears to be relatively high due to hybridization and gene flow mediated by long-distance wind pollination (Huang *et al.* 2002, Kanno *et al.* 2004, Chung *et al.* 2005). Species of *Quercus* and *Fagus* (subfamily Fagoideae) are mainly wind-pollinated whereas *Castanopsis* and *Lithocarpus* (subfamily Castaneoideae) are mostly pollinated by generalist insects (Manos *et al.* 2001). Gene flow and introgression in these insect-pollinated plants may therefore be more local compared with their wind-pollinated relatives. Together with seed-mediated dispersal such gene flow is likely to be one of the most important factors maintaining high diversity within forests at both the genus and species levels.

Taxonomic delineation of species in regions with high genetic diversity can be a difficult task. On the other hand, many studies have shown that molecular methods can be used to address certain questions; for example a taxonomic discrepancy involving Eurasian beech (*Fagus*) has been resolved by using combined evidence from nuclear genes, morphology and the fossil record (Denk *et al.* 2002). Nuclear ribosomal DNA sequences have been used as markers to assess the molecular systematics of oaks (*Quercus*) in Italy where the diversity includes most of the European species and a series of hybrids and ecotypes (Bellarosa *et al.* 2005). For South-East Asian species of Fagaceae, molecular tools have so far been used to reconstruct phylogeographic history of *Lithocarpus* (Canon & Manos 2003) and to estimate genetic diversity of a *Castanopsis* species at the population level (Blakesley *et al.* 2004). Other than that little is known about the overall genetic and species diversity of Fagaceae in the tropical regions of Asia.

The objectives of this paper were, therefore, (1) to investigate the diversity of Fagaceae species in Khun Mae Kuong Forest, which is one of the most species-rich regions of northern Thailand, and (2) to characterize genetic diversity and relationships among these Fagaceae species using molecular markers based on inter-simple sequence repeats (ISSR) which require no prior knowledge of DNA sequence data.

MATERIALS AND METHODS

Plant materials

Native trees belonging to the genera *Castanopsis*, *Lithocarpus* and *Quercus* in a forested region of northern Thailand located at approximately 18.87° N, 99.14° E were selected for this diversity study. The area of investigation, called Khun Mae Kuong Forest, covers about 550 km^2 in Doi Saket district, Chiang Mai province. This is part of the state conservation programme Huai Hong Khrai Royal Development Study Centre established in 1982. Three different types of forests were selected, namely, hill-evergreen forest (sites KPA and KNK), hill-evergreen forest with pine (KBA and KRD), both at relatively high elevation (1000–1800 m), and dry-deciduous forest at altitudes below 800 m (KPS and KHH). At each site 5–30 m tall trees (over 10 years old) at 30–100 m apart were selected for this investigation, usually starting from the highest point in a forest downhill. The trees were labelled and local names were recorded together with their relative locations. Twigs with fully grown leaves, fruits (acorns) and flowers were collected for taxonomic identification and kept as voucher specimens at the Botany Department, Chulalongkorn University. Young leaves were collected and stored in plastic bags containing silica gel until DNA isolation. Taxonomic identification followed *Flora Malesiana* (Soepadmo 1972) and the revision of the family Fagaceae in the *Flora of Thailand* (Phengklai *et al.* 2006). Due to variation in plant phenology, specimens for taxonomic identification had to be collected during several visits, usually in the cool and dry season (December– February) to get flowers and a few months later, acorns. Some species, especially in *Lithocarpus*, flower later, i.e. in the wet season (May–August).

Molecular analysis

Total genomic DNA was extracted from dried leaf samples as in Anamthawat-Jónsson and Heslop-Harrison (1995), using DNA extraction buffer containing 2% (w/v) CTAB (hexadecyltrimethylammonium bromide), 2% (w/v) PVP (polyvinylpyrrolidone), 1.4 M NaCl, 20 mM EDTA, 100mM Tris-HCl pH 8.0, and 2% (v/v) 2-mercaptoethanol. The PCR-based fingerprinting method, ISSR by Zietkiewicz *et al.* (1994), was used to detect variable sites in the microsatellite regions of the genome. After an initial screening of 32 ISSR markers provided in primer set #9 from the Biotechnology Laboratory, University of British Columbia, Vancouver, Canada on two to three species from each genus, 10 primers were selected (Table 1). These primers produced a high number of distinct (polymorphic) bands in all genera and species tested.

The ISSR amplification was performed in a final volume of 30 µl containing 10–20 ng total genomic DNA, 200 µM each of dNTPs, 0.25 µM primer, 3.0 mM $MgCl_2$, 1 unit Taq DNA polymerase (Fermentas, USA) and 1× PCR buffer (also from Fermentas). The amplification reactions were performed in a thermocycler (Techne, UK) under the following conditions: an initial denaturation step at 94 °C for 3 min, 45 cycles of 94 °C for 45 sec, 52 °C for 45 sec and 72 °C for 90 sec, and the last extension step at 72 °C for 10 min. The amplification fragments were separated in a 1.7% agarose gel and the sizes of amplified bands were estimated using GeneRuler 100bp DNA Ladder Plus marker (Fermentas). The reproducibility of ISSR amplification was ensured throughout the experiments by repeating PCR reactions for every plant sample at least twice and using overlapping samples as reference in successive amplification experiments. Only reproducible and unequivocally scorable ISSR profiles were analyzed further.

Table 1 ISSR primer sequences, number of fragments scored and approximate size range (in base pairs) of the fragments resulted from each primer probing 68 samples from 24 species of Fagaceae in northern Thailand

Primer from UBC set #9		No. of fragments scored	Fragment size range (bp)
Code	Sequence*		
810	$(GA)_8T$	17	450–1500
825	$(AC)_8T$	19	500–1800
834	$(AG)_8YT$	16	500–1500
835	$(AG)_8YC$	10	450–1500
840	$(GA)_8YT$	10	500–1300
857	$(AC)_8YG$	23	450–2000
868	$(GAA)_6$	12	550–1800
873	$(GACA)_4$	26	600–2800
876	$(GATA)_2(GACA)^2$	21	400–1900
881	$(GGGTG)_3$	19	400–2000

* Y = pyrimidine

The ISSR amplification products were scored from each plant sample as present (1) or absent (0). A pair-wise similarity matrix was constructed from these scores using the Dice similarity coefficient (Dice 1945). A phenogram was constructed using NTSYS-PC version 2.1 (Rohlf 1998) based on unweighted pair group method with arithmetic average (UPGMA). To test reliability of the tree, bootstrap test was performed with 100 replicates using PHYLIP programs Seqboot, Restdest, Neighbor and Condense (Felsenstein 2004).

RESULTS

Plant identification and species diversity

The plants were identified to genus and species based on flower, fruit (acorn), leaf and other vegetative and field characters. Species diversity of Fagaceae at Khun Mae Kuong Forest is shown in Table 2. From 146 trees examined in this study, 30 different species were identified. Three genera were found among these samples, namely, *Castanopsis*, *Lithocarpus* and *Quercus*. They were often recognizable in the field after the following features (Figure 1):

(1) *Castanopsis*: leaves serrate or entire; inner bark smooth; inflorescence erect (flower clusters upright); many stamens; fruits usually covered by spiny cupules

(2) *Lithocarpus*: leaves usually entire; inner bark with narrow ridges; inflorescence erect; six stamens; fruits mostly or partly covered by cupules, not spiny

(3) *Quercus*: leaves usually serrate; inner bark with narrow ridges; male catkins pendulous; six stamens; fruits partly covered by cupules, not spiny.

Table 2 Species diversity in Khun Mae Kuong Forest and number of trees examined and samples used in the DNA analysis

Plant species	No. of trees from each species at each site (No. of samples analyzed by ISSR)					
	Hill-evergreen forest		Hill-evergreen forest with pine		Deciduous dipterocarp forest	
	KPA	KNK	KBA	KRD	KPS	KHH
C. acuminatissima (Blume) A.DC.	-	12 (7)	3 (2)	3 (2)	-	-
C. argentea (Blume) A. DC.	1 (1)	1 (1)	-	1 (0)	-	-
C. armata (Roxb.) Spach	1 (0)	1 (1)	-	-	-	-
C. calathiformis (Skan.) Rehder & Wilson	3 (1)	2 (2)	-	-	-	-
C. cerabrina (Hickel & A. Camus) Barnett	1 (0)	1 (0)	-	1 (0)	-	-
C. crassifolia Hickel & A. Camus	1 (1)	1 (0)	-	-	-	-
C. diversifolia (Kurz) King & Hook.f.	4 (1)	2 (2)	-	-	-	-
C. echinocarpa A. DC.	-	-	1 (0)	1 (0)	-	-
C. ferox (Roxb.) Spach	4 (1)	1 (1)	-	1 (0)	-	-
C. fissa (Champ) Rehder & Wilson	2 (1)	-	-	-	-	-
C. indica (Roxb.) A. DC.	6 (2)	-	-	-	-	-
C. tribuloides (Sm.) A. DC.	-	-	-	8 (7)	-	-
L. ceriferus (Hickel & A. Camus) A. Camus	-	-	1 (1)	1 (0)	-	-
L. elegans (Blume) Hatus ex Soepadmo	-	1 (0)	1 (0)	1 (0)	4 (1)	-
L. harmandianus (Hickel & A. Camus) A. Camus	1 (1)	1 (1)	3 (3)	-	-	-
L. polystachyus (A. DC.) Rehder	-	-	1 (0)	2 (1)	-	-
L. recurvatus Barnett	-	-	1 (1)	-	-	-
L. sootepensis (Craib) A. Camus	-	-	2 (1)	2 (1)	2 (1)	-
L. vestitus (Hickel & A. Camus) A. Camus)	1 (0)	1 (1)	-	-	-	-
Q. brandisianus Kurz	-	-	3 (3)	2 (1)	4 (0)	-
Q. fleuryi Hickel & A.Camus	-	-	-	-	-	1 (1)
Q. helferianus A. DC.	-	-	-	-	2 (0)	-
Q. kerrii Craib	-	-	4 (1)	-	9 (1)	9 (5)
Q. kingianus Craib	-	-	2 (2)	-	-	2 (2)
Q. lenticellatus Barnett	-	-	-	1 (0)	-	1 (0)
Q. lineatus Blume	-	-	-	-	-	3 (2)
Q. mespilifolius Wall. ex DC.	-	-	-	-	4 (2)	2 (1)
Q. myrsinaefoius Blume	-	-	1 (0)		-	-
Q. quangtriensis Hickel & A. Camus	-	-	-	-	-	5 (0)
Q. rex Hemsl.	-	-	-	-	-	2 (1)
Total number of trees (146)	25 (9)	24 (16)	23 (14)	24 (12)	25 (5)	25 (12)

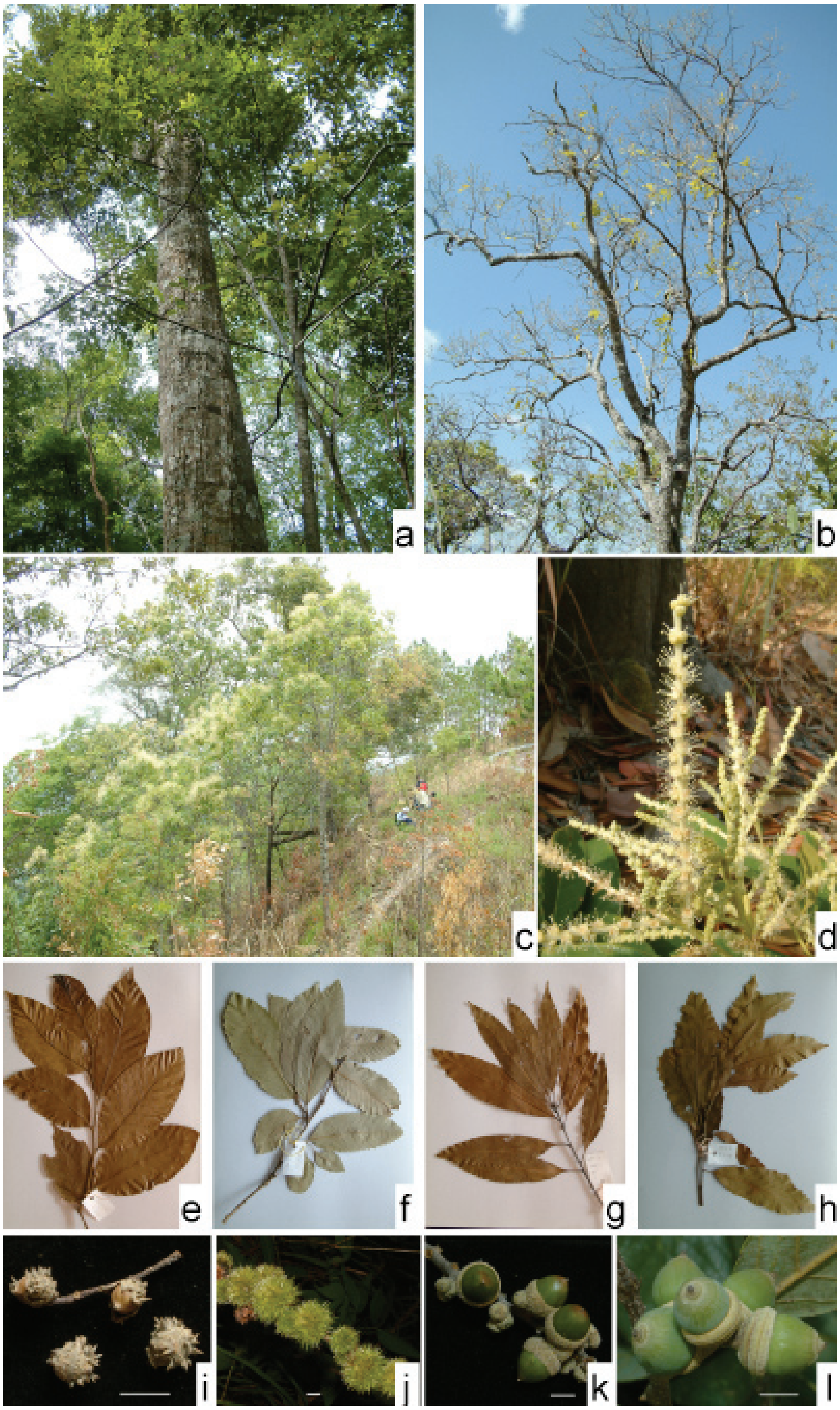

Figure 1 Fagaceae species at Khun Mae Kuong Forest at Doi Saket district, Chiang Mai province: examples of morphological characteristics used in taxonomic classification in this study. See list of species names and authorities in Table 2. Scale bars in Figures (i) – (l) represent 1 cm. (a) *Castanopsis acuminatissima*: tree no. NK14, October 2003; (b) *Quercus kerrii*: tree no. BA16, May 2004, showing newly formed leaves; (c) *Lithocarpus elegans*: tree no. RD1 and other trees of the same species, May 2004, showing synchronous flowering; (d) *C. tribuloides*: tree no. RD4, April 2003, showing erect inflorescences and numerous stamens, characteristics of *Castanopsis*; (e) *C. diversifolia*: tree no. NK4, showing elliptic shaped leaves with entire margin; (f) *C. calathiformis*: tree no. PA25, showing elliptic-oblong leaves with serrate margin; (g) *L. hermandianus*: tree no. NK8, showing narrow elliptic leaves with entire margin; (h) *Q. lineatus*: tree no. HH2, showing elliptic shaped leaves with serrate margin; (i) *C. acuminatissima*: tree no. NK15, showing acorns, completely enclosed cupule with sparsely spines; (j) *C. indica*: tree no. PA4, showing acorns, completely enclosed cupule with densely spines; (k) *L. hermandianus*: tree no. NK8, showing acorns, partially enclosed cupule with alternate lamentas; (l) *Q. kerrii*: tree no. BA15, showing acorns, partially enclosed cupule with annular lamentas.

Among these three genera, *Castanopsis* was found most frequently in hill-evergreen forests, *Quercus* was most common in deciduous forests at low altitudes, while *Lithocarpus* was found scattered in a wide variety of forest types. *Castanopsis* and *Quercus* mostly occupied separate locations and habitats. In the *Quercus*-rich deciduous forests none of the Fagaceae trees examined were identified as *Castanopsis*. In the hill-evergreen forests there were no *Quercus* trees among the samples except at the hill sites with pine. Evergreen species of *Lithocarpus*, such as *L. elegans* and *L. harmandianus*, were found in the evergreen forests but some species such as *L. ceriferus* and *L. sootepensis* were in deciduous and pine-evergreen forests together with several species of *Quercus*.

Out of 30 species found at Khun Mae Kuong Forest the genera *Castanopsis* and *Quercus* had similar numbers of species, i.e. 12 and 11 species respectively but *Lithocarpus* had only seven species. High species diversity within a site was also observed especially in hill-evergreen forests, whereby 11–12 species were identified from 23–24 trees in each site investigated. Several species in this collection were represented by only one individual. Nevertheless, certain species were more common than others; for example, *C. acuminatissima* was clearly the most common species in hill-evergreen forests and at one site (KNK) 12 out of 24 trees belonged to this species alone. *Quercus kerrii* was common in dry-deciduous forests (18 out of 50 trees) and tended to form large stands.

About half of the plants in this study were identified to species using both leaves and acorns, but the other half were identified either after acorn or leaf and/or flower. Taxonomic description of all 30 species under study was made based on both field and herbarium specimens (Chokchaichamnankit 2005). The most common species in each genus was classified after the following morphological characteristics:

(1) *Castanopsis acuminatissima* (Blume) A. DC. Tree (Figure 1a), 10–40 m high, 60–250 cm girth. Bark greyish brown to dark brown. Leaf elliptic oblong to oblong lanceolate, 6–13 by 2–4 cm. Base obtuse to slightly oblique. Apex caudate, acuminate or cuspidate. Margin serrate on the upper half. Acorn (Figure 1i) sessile, globose or ovoid, 1–1.5 by 0.8–1.3 cm, on erect infructescence 9–12 cm long. Cupule completely enclosed but not fused with the nut, skin covered sparsely with simple and curved beyond the base spines, pubescent then glabrescent, dehiscent. Nut one per cupule, ovoid, ca. 1 cm diameter, glabrous.

(2) *Quercus kerrii* Craib. Tree (Figure 1b), 5–40 m high, 50–150 cm girth. Bark blackish brown. Leaf oblong, ovate oblong to lanceolate, 10–24 by 3–10 cm. Base obtuse. Apex acute. Margin serrate. Lamina subcoriaceous, densely pubescent especially on lower surface, then glabrescent except along midrib and nerves on the lower surface. Acorn (Figure 1l) sessile, obovoid, flatted top, 0.5–1.1 by 0.5–2.1 cm, on erect infructescence 5–10 cm long, acorn free, 3–10 acorns in each infructescence. Cupule obconical-shaped, enclosed at the same level of the top of nut. Skin composes of grey to brown hairy lamentas, set in 5–10 fine annulars or lamellae. Nut one per cupule, flatted to minutely retuse apex, ca. 1.5 cm diameter, brown indumentum, then glabrescent.

(3) *Lithocarpus harmandianus* (Hickel & A. Camus) A. Camus. Tree (Figure 1c), 5–20 m high, 50–150 cm girth. Bark greyish. Leaf (Figure 1g) oblong, elliptic-oblong to obovate, 11–22 by 4–10 cm. Base slightly cuneate, oblique. Apex acute, acuminate. Margin entire. Acorn (Figure 1k) sessile, conical, 2–3 by 1.2–2 cm, on erect and without sub-branch infructescence, 10–25 cm long, acorn fused or addnate near base (some free), indehiscent. Cupule cup-shaped, enclosed up to one third of the nut. Skin composes of alternate and greyish brown lamentas, fused through the inner part completely. Nut one per cupule, conical, ca. 1.5 cm diameter, dull green indumentum, glabrescent.

Molecular diversity inferred from ISSR

ISSR polymorphisms were examined in 68 samples (individual trees) from 24 species (Table 2) using the 10 most polymorphic primers (Table 1). These ISSR primers generated 173 bands, ranging in size from 400 to 2800 bp and corresponding to an average of 17 bands per primer. A phenetic tree was constructed from these data (Figure 2). The number of individual trees in each species examined was variable, from one to 11 and this was because species

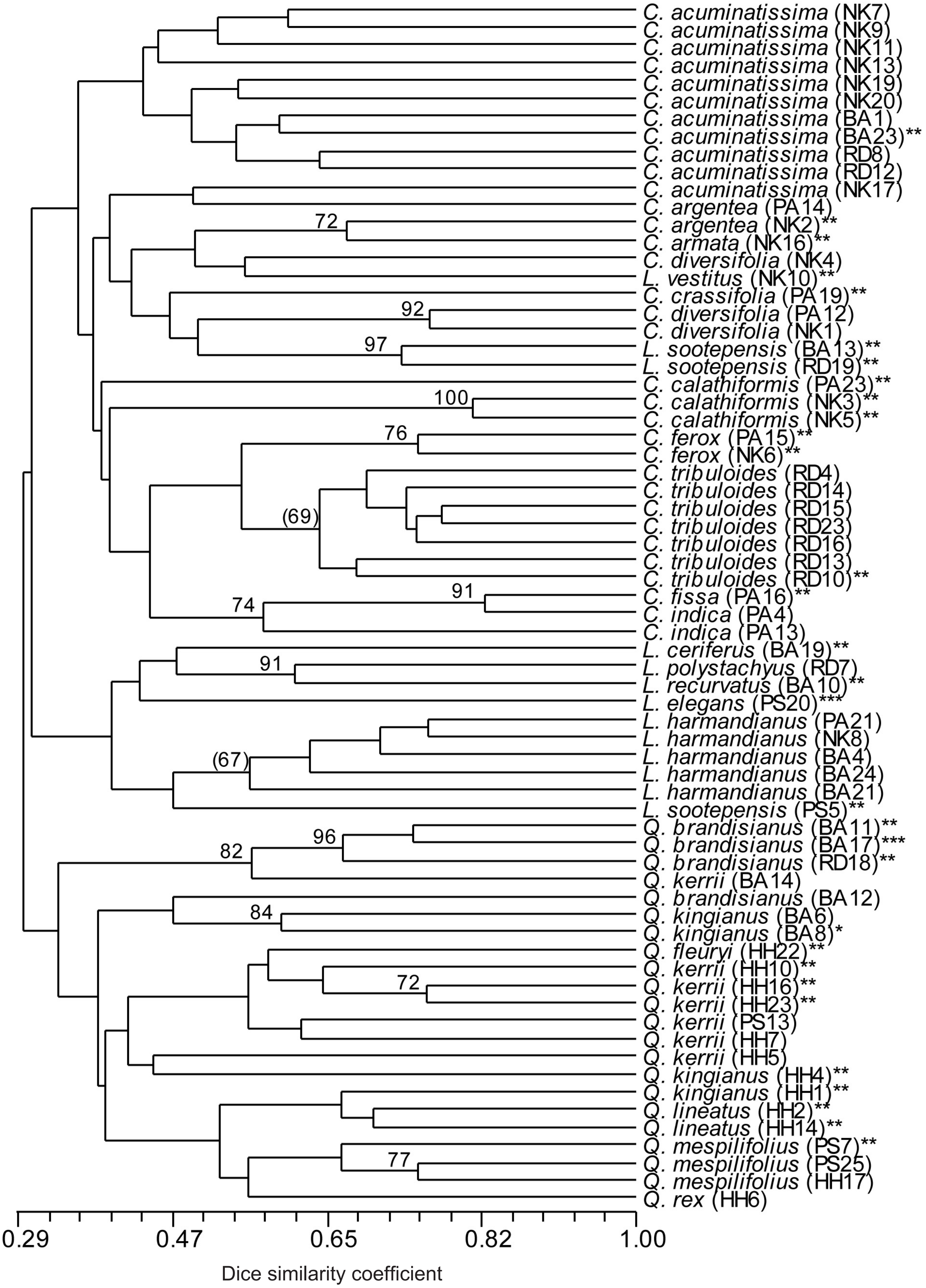

Figure 2 Molecular relationships in Fagaceae at Khun Mae Kuong Forest based on inter-simple sequence repeats (ISSR). The phenogram was constructed from 68 samples of 24 species (see list of species and tree numbers in Table 2). The horizontal scale shows Dice similarity coefficient. The plants were identified to species using leaves and acorns, except where indicated with *(acorns only), **(leaves only) and ***(leaves and flowers).

classification was performed after the trees were randomly selected for the species diversity study.

The ISSR results showed that the genera *Castanopsis*, *Lithocarpus* and *Quercus* were relatively well separated (Figure 2). Although the separation was not supported by bootstrap analysis the genus *Quercus* formed a distinct group while *Castanopsis* and *Lithocarpus* appeared to be more related to each other. Three *Lithocarpus* samples were included in the *Castanopsis* group and this could be the case of wrong classification as these samples were identified to species level based on leaf characters only. Leaf morphology is more variable in *Castanopsis* than in other genera (Phengklai *et al.* 2006) and, therefore, acorns are required to make identification of these Fagaceae samples reliable. Nevertheless, there could be genetic reasons behind this diversion. The sample NK10, which was the only sample of *L. vestitus* analyzed, appeared to be a polyploid tree (Chokchaichamnankit 2005), and this may have affected the plant morphology and consequently the taxonomic identification. The other two *Lithocarpus* samples which were included in the *Castanopsis* group, i.e. BA13 and RD19 (trees from different sites), both belonged to *L. sootepensis*. This is a new species recently described using the type specimen from Doi Sootep in Chiang Mai province (Phengklai 2004). There is no other molecular or chromosomal data on this species other than that found in the present study.

The grouping within species (where there was an adequate number of individuals) was also in congruence with the taxonomic classification (Figure 2). Ten out of 11 samples of *C. acuminatissima* were grouped together, especially those from the hill-evergreen forest KNK. All seven samples of *C. tribuloides* were grouped together and these trees were from the hill-evergreen forest with pine KRD. All five samples of *L. harmandianus* were also grouped together. All seven samples of *Q. kerrii*, mainly from the dry-deciduous forest KHH, also formed a distinct group separated from other *Quercus* species. Grouping within other species was not apparent as too few samples per species were available. However, several sets or pairs of samples were supported by the bootstrap analysis. Some of these sets consisted of samples belonging to the same species and from the same forest, for example *C. calathiformis* (NK3 and NK5), *Q. kingianus* (BA6 and BA8) and *Q. kerrii* (HH16 and HH23). Other sets consisted of samples of the same species from different sites, for example *C. diversifolia* (PA12 and NK1), *C. ferox* (PA15 and NK6), *L. sootepensis* (BA13 and RD19), *Q. brandisianus* (BA11, BA17 and RD18) and *Q. mespilifolius* (PS25 and HH17). The ISSR analysis indicated that trees belonging to the same species were more related to each other regardless of location or forest type.

DISCUSSION

Species diversity and forest resources

There are two main types of forests in Thailand, namely, evergreen and deciduous forests. In the northern regions of Thailand, the evergreen forest is represented mainly by hill-evergreen forest on the highland parts (above 1000 m), with trees of the family Fagaceae forming at least 50% of the main canopy layer, semi-evergreen forest where rainfall is between 100–200 cm, and pine forest on the highlands with poor soil. The deciduous forest, which is commonly found throughout the country, is broadly divided according to species composition into mixed-deciduous forest (with and without teak) and dry dipterocarp forest in dry areas where rainfall is below 100 cm and the soil condition is infertile and sandy. The selected study area at Khun Mae Kuong in Chiang Mai province includes both Fagaceae-rich hill-evergreen and deciduous forests.

The present study has shown that different forest types support different Fagaceae species and this seems to depend on several factors including elevation, habitats and level of disturbances. *Castanopsis* dominates well preserved hill-evergreen forests with closed canopy whereas *Lithocarpus* is found in more open and dry habitats at various elevations. Fagaceae, which is the main component of hill-evergreen forests, is losing ground to pine especially in areas with thin soils and frequent fires. It is, therefore, possible that widespread or highly adaptable species could establish themselves readily in such habitats. *Lithocarpus* is indeed prevalent in hill-evergreen forests with pine. *Quercus*, on the other hand, is found essentially in dry-deciduous forests. Dry-deciduous forests occupy low elevation sites that have little or no groundwater input during the dry season and the soil is often too thin to maintain water reserves.

The natural vegetation in such areas is often referred to as dry dipterocarp forest because it is frequently dominated by major hardwood trees of Dipterocarpaceae (*Dipterocarpus* and *Shorea*). These forests have a poorer canopy structure than other lowland forests and the tree species diversity is also much lower (Gardner *et al.* 2000, Santisuk 2005). A few species of Fagaceae are often present especially deciduous *Quercus* species. In areas with a long history of logging and fires a large part of the vegetation today is regenerated, forming secondary vegetation. *Quercus* is one of the trees that can form a good size stand after such disturbance.

Forestry in Thailand has undergone several changes including commercial logging which was the major type of forest exploitation from the 1940s to early 1960s. Since the late 1980s, Thailand has entered the conservation phase as the people have developed awareness of the adverse effects of forest exploitation. Whatever is remaining must be kept for conservation purposes and many areas are now closed to allow regeneration of forest trees (Santisuk 2005). Fagaceae species are among the most important trees for making the recovery of forests in northern Thailand a successful venture (Phengklai *et al.* 2005).

Species and genetic diversity

The present study has confirmed that northern Thailand, as represented by Khun Mae Kuong Forest in Chiang Mai province, preserves high species diversity with respect to Fagaceae. South-East Asia, Indochina in particular, maintained the greatest assemblage and most primitive forms of *Castanopsis* and *Lithocarpus* as well as *Quercus*, subgenus *Cyclobalanopsis* (Soepadmo 1972). Many of these species, especially in the genus *Castanopsis*, form part of the montane forest distribution east of Himalaya, including eastern Nepal, north-eastern India, northern Myanmar, northern Thailand, southern China and the western part of Indochina. Among the *Castanopsis* species, *C. acuminatissima* is considered to be the most widespread in South-East Asia (Barnett 1944, Gardner *et al.* 2000). Other species of *Castanopsis* identified in this study are not as common. The genera *Quercus* and *Lithocarpus* are also known to be highly diversified in South-East Asia and occur in the same range of distribution as *Castanopsis*. However, *Quercus* reaches much further north and east into Korea and Japan while *Lithocarpus* extends far south into the Malay Peninsula, Indonesia and coastal East Asia (Barnett 1944, Soepadmo 1972). A number of Fagaceae species are endemic to Thailand (Phengklai 2004) including *L. sootepensis* and *Q. lenticellatus* found in this study.

In an attempt to understand the species diversity of Fagaceae in Khun Mae Kuong Forest, we examined diversity at the molecular level by analyzing genome-wide ISSR. The molecular results are in good agreement with the taxonomic classification as the genera and most species are relatively well separated. Furthermore, the study has provided indication that certain species may have been misidentified or a taxonomic revision should be considered. Further molecular investigation will be necessary especially sequence-based analyses of nuclear and chloroplast genes. For example, sequence variation within the chloroplast *trn* regions has been widely used in phylogenetic and phylogeographical analyses of *Quercus*, *Castania* and *Castanopsis* (Petit *et al.* 1997, Kanno *et al.* 2004, Cheng *et al.* 2005, Magni *et al.* 2005, Lang *et al.* 2006). On the other hand, variation in the intergenic spacer region between the *rbcL* and *atpB* genes in the chloroplast genome was found to be more suitable for resolving the phylogeny of *Lithocarpus* than the various spacer regions in the *trn* genes (Canon & Manos 2003). Nuclear ribosomal DNA sequences encoding the 5.8S RNA and the flanking internal transcribed spacers (ITS1 and ITS2) were used successfully with samples of *Quercus* (Bellarosa *et al.* 2005) and these sequences were considered to be more species-dependent, even among widespread *Lithocarpus* species (Manos *et al.* 2001, Canon & Manos 2003). Dominant multilocus markers, such as ISSR used in the present study, are particularly useful for an initial examination of the partitioning of genetic variation within species or for locating centres of genetic diversity (Newton *et al.* 1999). However, they cannot determine specific variation at a single locus which is required for resolving phylogenetic relationships at species level or higher taxonomic status.

Chromosome-based methods are also highly applicable. A molecular cytogenetic mapping of 18S-25S and 5S nuclear ribosomal genes on 15 Fagaceae species from Khun Mae Kuong Forest (Chokchaichamnankit 2005) supports the taxonomic classification of this group of Fagaceae species. This ribosomal gene mapping is particularly useful for resolving taxonomic discrepancies as ribosomal gene maps tend to be species-specific. It would, therefore, be interesting to study *L. sootepensis* using such approach as two out of three samples of this species in this ISSR study have clustered within the genus *Castanopsis*. As mentioned earlier, more taxonomically informative specimen such as acorns may be sufficient. However, some genotypes, especially interspecific hybrids or polyploids, may never produce acorns. The only sample of *L. vestitus* in this ISSR study has also grouped within *Castanopsis*. Partial ribosomal gene map has shown that this tree is originally a tetraploid (Chokchaichamnankit 2005). This tree has never produced flowers since the study began in 2001 and the other tree belonging to this species has produced flowers that never developed into acorns. They both are most likely sterile.

The ISSR method is useful in providing background necessary for the effective management and conservation of forest trees. The technique is based on the presence of various polymorphic microsatellite motifs in the plant genomes (Lagercrantz *et al.* 1993, Zietkiewicz *et al.* 1994). As the polymorphism is probably genome-wide, it should be suitable for phenetic analysis. This method is simple, inexpensive and has proved to be applicable for taxonomic inference and genetic diversity assessment in a wide range of organisms. ISSR markers have been used for characterizing taxonomic diversity in *Nothofagus* of the Southern Hemisphere (Mattioni *et al.* 2002), estimating loss of genetic diversity in an endangered pine species in China (Zhang *et al.* 2005). In addition when geographical coordinates and genetic differentiation are combined, such markers can detect changes in the gene flow in forest trees (Lu *et al.* 2005). Tree species are becoming the focus of increasing conservation concern with some 9000 species threatened globally (Newton *et al.* 1999). Studies of genetic diversity, especially intraspecific and interspecific variation, can contribute to the development of conservation strategies by identifying units for conservation.

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