



CHAPTER I

INTRODUCTION

Fagaceae (Beech family) dominates forests in the temperate, seasonally dry regions of the Northern Hemisphere, with a centre of diversity found in tropical Southeast Asia, particularly at the generic level (Manos et al., 2001). In Thailand, this family comprises four genera (Forman, 1964; Smitinand, 1980; Gardner et al., 2000): *Castanopsis* (D. Don) Spach. (chestnut, mostly evergreen, ca. 100 species in tropical and subtropical Asia), *Lithocarpus* Blume (stone oak, mostly evergreen, 100 - 300 species mainly in Asia), *Quercus* L. (oak, mostly deciduous, 300 - 500 species, mainly in the northern temperate zone with an extension into the subtropics and tropics) and *Trigonobalanus* Forman (two species in Asia, one of which is in Thailand, and one species in South America). Altogether there are 40 - 60 species of Fagaceae in northern Thailand, or 5 - 10 % of the total world species number in this family. Several species of Fagaceae are utilized by the locals for edible nuts, which are very much sought after especially from *Castanopsis* (e.g. *C. diversifolia* and *C. acuminatissima*). Fagaceae from this region is generally not good timber but excellent firewood. It has also been used for growing Shiitake mushrooms. As part of the nation-wide forest restoration effort, work is well underway to establish new Fagaceae trees in the headwater catchment's areas especially in northern Thailand.

Little is known about the level of genetic diversity among species of Fagaceae in Southeast Asia, although this region is central to the species distribution and diversity of this family (Soepadmo, 1972). Based on studies of Fagaceae in the temperate regions (see Chapter 2), the genetic diversity in this family is expected to be substantial, due to morphological variation, ecological adaptation, clinal differentiation, hybridisation, gene flow and introgression. Such complex diversity pattern has made taxonomic delineation of species a difficult task. However, in recent years the methods of molecular genetics have proven useful for addressing taxonomic

questions and expanding our knowledge about the diversity and the mechanisms that maintain diversity in space and time. As many studies have shown, for example a taxonomical 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 European species and a series of hybrids and ecotypes (Bellarosa et al., 2005). Chloroplast DNA sequence variation has been used to reconstruct phylogeography of the Southeast Asian stone oaks (*Lithocarpus*), from Yunnan to Borneo (Cannon and Manos, 2003).

As a result of widespread deforestation and over exploitation, many tree species have recently become the focus of increasing conservation concern. Studies of genetic diversity, especially intraspecific and interspecific variation, can contribute to the development of conservation strategies, by identifying units for conservations. Two types of units for conservation have been defined (Newton et al., 1999): evolutionarily significant unit (ESU) and managing unit (MU). The ESU requires knowledge about phylogeographic pattern or historical population structure of a species, whereas the MU may be more suitable in situations where current population structure is more accessible and where the molecular facilities can only support small-scaled studies like this proposed project. In the absence of phylogeographic analysis, it might be impossible to differentiate between ancient polymorphism and current patterns of gene flow as the cause of such differentiation. Despite this, the identification of MUs can be of practical importance for informing the transfer of germplasm within and between regions, as can occur in reforestation or restoration activities like that of the Beech family in northern Thailand. Management units could potentially be defined on the basis of any population differentiation detected. For example, the RAPD analysis of Spanish cedar (*Cedrela odorata*) in Costa Rica (Gillies et al., 1997), or the mitochondrial (mt)DNA variation observed in Scots pine (*Pinus sylvestris*) in western Europe (Sinclair et al., 1999) and beech (*Fagus crenata*)

in Japan (Tomaru et al., 1998; Davis and Shaw, 2001), could be used to define separate MUs for these species.

Molecular approaches are of great value to conservation efforts by providing a tool for measuring and managing genetic diversity and for investigating the processes that influence it. This is the main aim of my project – to identify molecular variation and genetic/genomic relationships among Fagaceae species for conservation purposes. More importantly, the methods chosen here are mainly based on the nuclear genome, which is bi-parentally inherited, and should therefore be even more powerful than organelle-based markers, especially with this group of Fagaceae that may be as effectively dispersed by seed as with pollen. Greater use of nuclear DNA markers holds particular promise for future studies because much of the nuclear genome is still largely unexplored (Schaal et al., 1998; Newton et al., 1999).

I intended to characterize overall genetic diversity of Fagaceae in one of the species-richest regions in northern Thailand. Before we can sort out species taxonomically and manage our tree genetic resources correctly, we must understand the genetic diversity and get an overview of the species relationships and the evolution of this plant group. This project aimed at using molecular approaches, specifically restriction fragment length polymorphism (RFLP) of nuclear ribosomal genes (rDNA) and inter simple sequence repeat (ISSR), to characterize genetic diversity and genetic relationships among Fagaceae species from northern Thailand. Mutation in the nuclear genome is believed to occur at a higher rate than in the chloroplast or mitochondrial genomes, and is thus more likely to reveal population genetic structure in a small geographical region as in this study.

In order to supplement the conservation programmes, it is important to know the structure and behaviour of chromosomes and genomes to elucidate evolutionary potential of a population and also to underpin the structural rearrangements. The genomic and species relationships in Fagaceae will be investigated using karyotype analysis and molecular cytogenetic approaches that have rarely been applied to tree

species. Genome analysis by karyotyping and *in situ* hybridisation can shed light onto mechanisms behind the genetic variation of a species. The oak (*Quercus*) species examined to date are all diploid ($2n = 2x = 24$) and having very similar karyotypes (Schwarz, 1964; Ohri and Ahuja, 1990; Zoldos et al., 1999). Other species of *Quercus* and its related genera have not been examined cytogenetically. Two ribosomal gene families, 18S-25S rDNA and 5S rDNA, will be mapped on chromosomes, with the aim to differentiate species and genera, to characterize species relationships and to map molecular and chromosomal changes that may have occurred during the evolution of this Fagaceae family.

Objectives

1. To characterize species diversity of Fagaceae in a selected region in northern Thailand.
2. To investigate genetic diversity among these Fagaceae species using molecular approaches.
3. To describe cytotaxonomic features of this plant group and analyze genetic and genomic relationships among these tree species using molecular cytogenetic methods.