

FOREST RESTORATION PLANTING IN NORTHERN THAILAND

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Introduction

Deforestation is one of the most serious threats to biodiversity in developing countries. It causes floods, soil erosion and disease (owing to the loss of organisms that help to control vector populations), degrades watersheds and destroys wildlife habitats. Deforestation may extirpate populations and reduce genetic diversity within populations (Kanowski 1999). In northern Thailand, large areas in national parks and wildlife sanctuaries have been deforested. Government and non-governmental organizations and local communities must all be involved in the reforestation and restoration of these forests.

Thailand's forest cover was about 53% in 1950 (Bhumibhamon 1986), but is now 22.8% or 111,010km² (FAO 1997). These figures, however, do not distinguish between plantations and natural forest. Thailand's natural forest cover is unofficially estimated to be 20% (Leungaramsri & Rajesh 1992). The rate of forest loss peaked in 1977 and fell to its lowest level in 1989 when commercial logging was banned. National parks and wildlife sanctuaries cover 14.2% of the country but large areas of these are deforested and fragmented (Bontawee *et al.* 1995). Habitat loss affects plant species in many ways, for example by reducing population sizes, altering the density of reproductive individuals, reducing reproductive success, increasing isolation and reducing genetic diversity. Founder effects, genetic drift and restricted gene flow increase inbreeding, genetic isolation and divergence (Bawa 1994; Dayanandan *et al.* 1999; Rosane *et al.* 1999). Such processes may also influence the evolutionary potential of populations and species, particularly if adaptive genetic variation declines to a point where populations can no longer adapt to changing environmental conditions (Young *et al.* 1993).

The Forest Restoration Research Unit (FORRU)

Forest restoration involves planting native tree species and extending forest boundaries by artificial and natural regeneration (Bawa *et al.* 1990). Government and non-governmental organizations and local communities must all be involved in restoring forests. Historically, reforestation often meant establishing single-species plantations, mainly of pines and eucalypts, which are of little value for wildlife conservation or watershed protection. Since 1993, Thailand has implemented various reforestation projects to celebrate the Golden Jubilee of King Bhumibol Adulyadej. These have promoted the use of a wide range of native forest tree species. However, the implementation of this change in planting policy has been hindered by a lack of information on native species suited to planting in deforested areas (Elliott *et al.* 1997).

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The Forest Restoration Research Unit (FORRU) of Chiang Mai University was established in November 1994 to tackle some of the technical problems of re-establishing natural forest ecosystems on degraded sites in national parks and wildlife sanctuaries in northern Thailand (Elliott *et al.* 1995). The unit is a joint initiative between the Department of Biology in the University's Faculty of Science and Doi Suthep-Pui National Park (under the Royal Forest Department). FORRU is located at the headquarters of Doi Suthep-Pui National Park in Chiang Mai province.

A framework species method, which relies on selected fast-growing tree species, has been tested in the conservation areas of northern Thailand. FORRU aims to develop effective methods to complement and accelerate natural regeneration on deforested sites in conservation areas. Specific objectives include:

- i) Developing tools to study the restoration of natural forest ecosystems, such as a seedling identification handbook, seedling herbarium and database of seed, fruits and seedling morphology;
- ii) Understanding ecological processes of natural forest regeneration to determine ways in which these processes can be accelerated;
- iii) Identifying tree species that can be planted to complement natural seedling establishment;
- iv) Developing appropriate methods to propagate such tree species and test their performance after planting in the field; and
- v) Training interested groups in restoration techniques developed by the unit.

Framework species method

The framework species method (Goosem & Tucker 1995; Lamb *et al.* 1997) was originally developed in Australia for forest restoration. It relies on selecting fast-growing tree species with dense crowns, which can rapidly shade out competing weeds and attract seed-dispersing wildlife, particularly birds and bats. Framework species should also be easy to propagate in nurseries.

From initial work on more than 350 native tree species in Doi Suthep-Pui National Park, FORRU identified a number of potential framework species (Table 1). Thirty-one framework species were selected and planted to catalyse the recovery of plant and animal diversity in degraded forest areas. The selected tree species were grown in a nursery and planted out in experimental plots to determine whether the framework species method is applicable in northern Thailand. Important groups of framework species include fig trees (*Ficus* spp., Moraceae), legumes (Leguminosae) and oaks and chestnuts (Fagaceae).

Table 1. List of some potential framework tree species

Family	Species
Leguminosae, Mimosoideae	<i>Albizia chinensis</i>
Leguminosae, Mimosoideae	<i>Albizia odoratissima</i>
Euphorbiaceae	<i>Balakata baccata</i>
Betulaceae	<i>Betula alnoides</i>
Euphorbiaceae	<i>Bischofia javanica</i>
Verbenaceae	<i>Callicarpa arborea</i> var. <i>arborea</i>
Fagaceae	<i>Castanopsis acuminatissima</i>
Fagaceae	<i>Castanopsis tribuloides</i>
Lauraceae	<i>Cinnamomum iners</i>
Leguminosae, Papilionoideae	<i>Dalbergia cultrata</i>
Urticaceae	<i>Debregeasia longifolia</i>
Ebenaceae	<i>Diospyros glandulosa</i>
Elaeocarpaceae	<i>Elaeocarpus braceanus</i>
Juglandaceae	<i>Engelhardtia spicata</i>
Leguminosae, Papilionoideae	<i>Erythrina stricta</i>
Leguminosae, Papilionoideae	<i>Erythrina subumbrans</i>
Theaceae	<i>Eurya acuminata</i> var. <i>wallichiana</i>
Moraceae	<i>Ficus altissima</i>
Moraceae	<i>Ficus benjamina</i> var. <i>benjamina</i>
Moraceae	<i>Ficus microcarpa</i> var. <i>microcarpa</i>
Moraceae	<i>Ficus subulata</i> var. <i>subulata</i>
Verbenaceae	<i>Gmelina arborea</i>
Proteaceae	<i>Helicia nilagirica</i>
Meliaceae	<i>Heynea trijuga</i>
Myristicaceae	<i>Horsfieldia amygdalina</i> var. <i>amygdalina</i>
Myristicaceae	<i>Horsfieldia thorelii</i>
Rhamnaceae	<i>Hovenia dulcis</i>
Lauraceae	<i>Litsea cubeba</i>
Euphorbiaceae	<i>Macaranga denticulata</i>
Magnoliaceae	<i>Manglietia garrettii</i>
Bignoniaceae	<i>Markhamia stipulata</i> var. <i>kerrii</i>
Meliaceae	<i>Melia toosendan</i>
Moraceae	<i>Morus macroura</i>
Nyssaceae	<i>Nyssa javanica</i>
Euphorbiaceae	<i>Ostodes paniculata</i>
Lauraceae	<i>Phoebe lanceolata</i>
Euphorbiaceae	<i>Phyllanthus emblica</i>
Rosaceae	<i>Prunus cerasoides</i>
Leguminosae, Papilionoideae	<i>Pterocarpus macrocarpus</i>
Fagaceae	<i>Quercus semiserrata</i>
Anacardiaceae	<i>Rhus rhesoides</i>
Sapindaceae	<i>Sapindus rarak</i>
Theaceae	<i>Schima wallichii</i>
Anacardiaceae	<i>Spondias axillaris</i>
Polygalaceae	<i>Xanthophyllum flavescens</i>

Fig trees

Fig trees produce an edible inflorescence known as a syconium (fig), which looks like a fruit and consists of a fleshy cup with a small orifice at one end closed by interlocking scales. Enclosed within the fig are hundreds of minute flowers which, after pollination by specialized fig wasps, develop into tiny fruits. Birds are attracted to feed in fig trees, where they simultaneously disperse seeds of other tree species, thus adding to tree species diversity in rehabilitated forest areas. About 20% of planted seedlings should be fig species in the framework species method (Elliott *et al.* 1998). At least 47 fig species are indigenous to northern Thailand (CMU Herbarium Database 2000). *Ficus subulata* var. *subulata* is one of

the framework species. It is a small evergreen tree, which branches densely and produces figs two years after germination. It grows in disturbed areas and mixed deciduous forests at elevations of 800–1600m. Seeds germinate within 20–60 days of sowing.

Legumes

Legumes are fast-growing species with wide crowns. Many have nodules on their roots that fix nitrogen. These enable them to grow well on degraded sites with low nutrient levels. Sixty-one leguminous tree species are indigenous to northern Thailand (CMU Herbarium Database 2000). For example, *Erythrina subumbrans* is a fast-growing deciduous tree with a wide-spreading crown. Its thorny stem protects young trees from herbivores. Seeds are dispersed by wind and birds. The species grows in evergreen and mixed evergreen-deciduous forests at elevations of 350–1700m, especially along stream valleys at lower elevations. It is one of the fastest-growing tree species investigated by FORRU.

Oaks and chestnuts

Oaks and chestnuts (Fagaceae) also produce densely spreading crowns, and their nuts attract seed-dispersing wildlife into reforested areas (Elliott *et al.* 1998). There are 40 species in the Fagaceae family in northern Thailand (CMU Herbarium Database 2000). For example, *Quercus semiserrata* is a large evergreen tree with a straight bole and dense crown. It is one of the fastest-growing species of Fagaceae. It grows in evergreen forests, pine forests and mixed evergreen-deciduous forests at elevations of 800–1700m (Elliott *et al.* 1998).

Other framework species

Many other tree species from other families can also be used as framework species if they have a high growth rate and produce fruits that attract seed-dispersers. For example, *Bischofia javanica* (Euphorbiaceae) has a very dense crown and produces good construction timber. It produces small fleshy fruits (drupes), which are especially attractive to birds. *Gmelina arborea* (Verbenaceae) is a fast-growing deciduous tree, which also produces drupes two to three years after planting. This species is widely used for pulp, floors, ceilings, furniture, carving, boats and tools. *Prunus cerasoides* (Rosaceae) is a medium-sized, fast-growing deciduous tree that produces edible red fruits attractive to birds. It is also popular because of its attractive flowers. *Melia toosendan* (Meliaceae) is another fast-growing, deciduous framework species.

Framework species plots

The first plots of framework species were planted in 1998, and the trees are now three-years old. Saplings of 30 species were 50–60cm tall when planted at a density of 500 saplings per rai (3125 per hectare). Various post-planting treatments have been tested, including fertilizer, mulching and weeding treatments. The data from these experiments are still being analysed. In general, however, we have observed that canopy closure begins by the end of the second growing season, and is nearly complete by the third growing season. Weeds are effectively shaded out and a rich leaf litter begins to accumulate. The planted trees catalyse an increase in species richness by enhancing natural regeneration and ground flora and bird communities (Table 2).

Table 2. *Trees more than one metre tall in plots planted in June 1998*

	Pre-planting survey May 1998		End of 1st growing season 1998		End of 2nd growing season 1999		End of 3rd growing season 2000	
	Control	Planted	Control	Planted	Control	Planted	Control	Planted
Naturally Established Trees								
Mean density (per ha)	265	138	509	276	562	371	722	626
Species	12	11	20	22	23	26	25	30
Planted Trees								
Mean density (per ha)	0	0	0	361	0	1,527	0	1,984
Species	0	0	0	7	0	24	0	28
Total								
Mean density (per ha)	265	138	509	637	562	1,898	722	2,610
Species ^{a)}	12	11	20	26 ^{a)}	23	45 ^{a)}	25	52 ^{a)}

^{a)} The total number of species is less than the sum of natural and planted species because some of the planted species also grew naturally in the planted plots.

Selecting superior mother trees

Seedling performance has been variable in the field. The seedlings produced by FORRU are usually propagated from seeds of only one or two mother trees. Clearly, this practice will narrow the genetic base of the restored forests. The ideal characteristics of superior planting material include high rates of germination, fast growth in the nursery and fast growth in degraded areas. In theory, large seeds should yield larger, more competitive seedlings with better performance. Most studies have demonstrated that large seeds have many advantages over small seeds. For example, large seeds usually have higher germination rates (Black 1959; Cideciyan & Malloch 1982; Wise 1982; Nizam & Hossain 1999), greater or more rapid emergence from deeper sowing depths and less stringent requirements for litter and herbaceous cover (Winn 1985). Large seeds also have lower mortality rates (Schaal 1980; Tripathi & Khan 1990; Bonfil 1998) and higher seedling growth rates (Seiwa 2000). As a result, large seeds may give rise to better competitors (Anderson 1971; Wulff 1986).

Small seeds, however, have a competitive advantage over large seeds because they germinate earlier and are dispersed over greater distances (Jackson 1981; Howe & Richter 1982). This increases the probability that seeds will reach a favourable microsite for germination. Seed weight, therefore, seems to play an important role in the process of dispersal and population recruitment. Nizam and Hossain (1999) and Sawaminathan and Sivagnanm (1999) reported that seed germination and seedling growth increased with greater seed mass. Seedlings of heavy-seeded species tend to survive longer when grown in the absence of any mineral nutrients, and are able to emerge from greater depths in the soil than seedlings of light-seeded species. Other studies, however, have not found any relationship between seed size and seedling performance (Cipollini & Stiles 1991; Rice *et al.* 1993), seed germination (Vaughton & Ramsey 1998; Eriksson 1999), seedling survival (Hendrix & Trapp 1992) or seedling growth (Dalan 1984; Marshall 1986).

Tree planting is one of many steps in forest restoration. One of its first requirements is that seedlings are of high quality. Such seedlings must have the ability to produce new roots rapidly, resume photosynthesis and continue growth (Burdett 1983). Seed quality usually refers to genetic, physical and physiological quality. Seeds with good physiological quality have high vigour and germination rates. Physical quality refers to seed size and infestation by pathogens. The advantages of good physical and physiological seed quality are improved storage characteristics, minimal seed wastage and uniform seedlings in the nursery. Genetic

quality refers to the inherent capacity of seeds to produce trees adapted to a given environmental condition (Turnbull 1995).

Factors that affect seedling production in nurseries include incomplete release of seeds from dormancy, pre-treatment methods, age, ripeness at collection and seed processing (Poulsen 1993). Patterns of genetic variation should be apparent in patterns of phenotypic variation (Bawa *et al.* 1990). Studies of flowering and fruiting phenology, morphology of adult trees, seed germination and early seedling performance in the nursery can indicate superior mother trees. Seeds for nursery propagation are usually collected from a limited number of mother trees, a practice that may narrow the genetic base and reduce allele frequencies.

Molecular markers in genetic studies

Molecular markers are being used increasingly to analyse the genetic structure of populations, gene flow, population viability and, ultimately, to quantify the effects of habitat fragmentation and guide conservation strategies. Microsatellites, or simple sequence repeats, have a strong discriminatory power (Chase *et al.* 1996; Dayanandan *et al.* 1997). Microsatellites consist of tandem repeats of short nucleotide sequences (Zhao & Kochert 1993). They are widely dispersed in eukaryotic genomes and are often highly polymorphic owing to variations in the number of repeated units (Bruford & Wayne 1993).

Microsatellites were first developed for mapping the human genome (Waber & May 1989; Brunel 1994; White & Powell 1997). Subsequently they were found to be applicable to plant genomes (Morgante & Olivieri 1993). In recent years, microsatellites have become a popular tool for genetic mapping and analysing mating systems, paternity and patterns of gene flow within and between plant populations. They may also prove to be useful for quality control in tree-breeding programmes, and for certifying genetically improved seed and planting stock.

In trees, the first microsatellites were developed for radiata pine (*Pinus radiata*) (Smith & Devey 1994). They have since been developed for many other species, including *Quercus* spp. (Dow *et al.* 1995; Barrett *et al.* 1997; Isagi & Suhandono 1997; Lexer *et al.* 2000), *Eucalyptus* spp. (Byrne *et al.* 1996), other *Pinus* spp. (Echt *et al.* 1996; Thomas *et al.* 1999), Norway spruce (*Picea abies*) (Pfeiffer *et al.* 1997) and *Larix* spp. (Khasa *et al.* 2000).

Microsatellites for tropical trees were first developed for *Pithecellobium elegans* (Mimosoideae) (Chase *et al.* 1996). Subsequently they have been developed for *Swietenia humilis* (White & Powell 1997; White *et al.* 2000), *Gliricidia sepium* (Dawson *et al.* 1997), *Shorea curtisii* and other dipterocarp species (Tokuko *et al.* 1998), *Symphonia globulifera* (Aldrich *et al.* 1998), *Carapa guianensis* (Dayanandan *et al.* 1999), *Caryocar brasiliense* (Rosane *et al.* 1999) and *Melaleuca alternifolia* (Rossetto *et al.* 1999). The application of microsatellites to population and conservation genetics of tropical forest trees is limited by a lack of DNA sequence information for many tropical tree species. However, microsatellite primers developed for one species can sometimes be used to detect polymorphisms in related species, thus minimizing the need for laborious cloning and screening procedures (Dayanandan *et al.* 1997).

FORRU's studies on forest genetics

We are carrying out an assessment of the usefulness of microsatellites in contributing to the selection of seed trees for our forest restoration programme. This work has initially focused on *P. cerasoides* (Rosaceae). We are studying the genetic diversity of this species in three

national parks in northern Thailand: Doi Suthep-Pui, Doi Intanon and Doi Angkang. At the same time, we are also studying the nursery and field performance of seeds from selected parent seed trees. Primer pairs of microsatellites that display a high degree of polymorphism have been used in the amplification reaction, including primers isolated from peach (*Prunus persica*) (Cipriani *et al.* 1999), sour cherry (*Prunus cerasus*) (Suzanne *et al.* 2000) and sweet cherry (*Prunus avium*) (Downey & Iezzoni 2000).

Concluding remarks

Preliminary results suggest that primers developed for peach, sweet cherry and sour cherry can be used for genetic studies in *P. cerasoides*. On the basis of results from microsatellite studies, nursery studies (e.g. seed germination, growth rate, health of seedlings) and studies in experimental plots, we hypothesize that a group of superior mother trees of *P. cerasoides* could be selected while still maintaining genetic diversity.

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