Part 2

Invited Papers

EX SITU CONSERVATION EFFORTS FOR *Neobalanocarpus heimii* IN MALAYSIA

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Introduction

Forest genetic resources are the genetically heritable component of intra-specific variability that is of actual or potential use to man. *In situ* conservation is the principal method for conserving forest genetic resources. International perspectives, as Roche (1994) notes, are important to striking a balance between national *ex situ* and *in situ* conservation efforts. For economically important plant species, it is also necessary to consider the relationship between conservation and utilization, and to recognize that biotechnology can play an important role in conservation programmes.

Benson (1999) outlines the key steps to follow when embarking on a conservation strategy that incorporates biotechnology applications (Figure 1). The feasibility of using biotechnology must be considered in terms of resources, expertise and specific training needs, cost and long-term maintenance. This paper addresses these issues in the context of *ex situ* conservation of *Neobalanocarpus heimii*, a highly endemic tree species confined to Peninsular Malaysia and Pattani in southern Thailand (Symington 1943; Ashton 1982). It is classified by IUCN as 'vulnerable' but not critically endangered (Chua 1999).

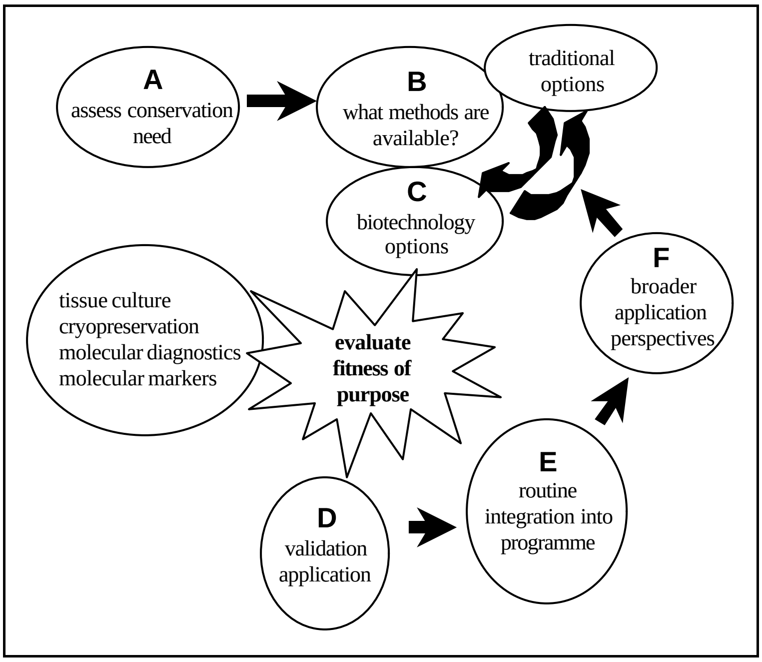

***Figure 1**. Integrating biotechnology into conservation projects. Source: Benson (1999).*

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Natural distribution

Descriptions of *N. heimii*, known locally as chengal, are given by Ashton (1982) in Flora Malesiana and Wong *et al.* (1994) in the Plant Resources of South-East Asia. The tree produces a heavy hardwood timber which is highly valued for its strength, durability and workability. It also produces a high-quality resin known as dammar. *N. heimii* is regarded by the timber trade as a 'primary hardwood', but the Malaysian government has banned its export in round log form. According to unpublished information of the Forest Department of Peninsular Malaysia, the largest specimen of *N. heimii* in Malaysia is in Pasir Raja Forest Reserve in Dungun, Terengganu. This tree is 65m tall, with a girth of 16.75m and a diameter at breast height of 5.33m.

According to Wong *et al.* (1994) and Chua (1998), *N. heimii* is extinct in Singapore and faces a high risk of extinction (or may already be extinct) in southernmost peninsular Thailand. In Peninsular Malaysia, *N. heimii* can still be found in protected areas in most states, except Perlis, Penang and Malacca (Figure 2). It is the second most dominant species in the Pasoh Forest Reserve in Negeri Sembilan, although it accounts for only 1% of all trees (Manokaran 1990). It is often found growing on undulating, well-drained areas with soils of average fertility (Ashton 1982; Wyatt-Smith 1987), but occurs less frequently at higher elevations. Symington (1943) reported that it was often found at low densities (fewer than five trees per hectare) in natural stands. Stands dominated by *N. heimii* usually have only a few small and intermediate trees. More recent surveys (Chin *et al.* 1997) suggest high endemism and localised but dense distribution of this species.

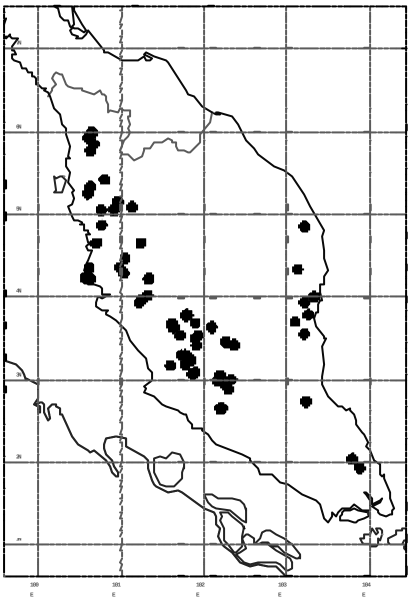

Figure 2*. The distribution of N. heimii in Peninsular Malaysia and the location of the study sites. Source: L. G. Saw.*

According to Wong *et al.* (1994), this species is much rarer now than it was early in the 20th century. Its endemism and limited distribution justify conservation measures to safeguard the remaining populations. In view of the demand for this and other timber species, the Malaysian government has taken steps to implement conservation measures and sustainable management practices in remaining forest areas. Most of areas populated by *N. heimii* in Malaysia's virgin jungle reserve system have been designated as research plots. The largest plot is in Balok Forest Reserve, Compartment 8 in Pahang (Saw & Raja Barizan 1991).

A great deal of experience in cultivating *N. heimii* has been gained since the era of gutta percha extraction around 1900–1913 (Ashton 1983; Appanah & Weinland 1994). This information is considered adequate for current planting efforts. The limited availability of seed sources, however, is still a constraint to conservation programmes for this species.

Genecology

The elucidation of population structures and patterns of gene distribution within ecosystems provides information that can be used to support *in situ* conservation efforts. A molecular knowledge of genetic diversity can facilitate germplasm collection and greatly assist in the decision-making processes of *ex situ* conservation (Benson 1999). An understanding of genetic structure and gene flow within plant populations in their natural distribution range is needed before any conservation plan can be prepared.

Konuma *et al.* (2000) found that the reproduction of *N. heimii* is mediated by a long-distance pollinator. They estimated a minimum average mating distance of 524m. According to Appanah (1979), bees from the genera *Apis* and *Trigona* are the main pollinators of *N. heimii* flowers. Any conservation efforts, therefore, should focus not only on the tree itself, but also on its mating and dispersal systems, including pollen and seed dispersal vectors. The disappearance of these vectors would reduce seed production and dispersal, and cause serious reproductive deficiencies.

Appanah (1979) also found that flower production in *N. heimii* peaks around the fourteenth to fifteenth day of flowering. He concluded that peak flowering events in *N. heimii*, as in *Shorea*, tend to occur in a single burst (see Phenology below). Such behaviour may maximize the opportunities for out-crossing and simultaneously increase the size of the potential pool for gene exchange (Chan & Appanah 1980). Owing to the fragmentation of forest areas, the establishment of seed production areas and seed orchards for *N. heimii* has long been mooted by tree improvement and tree breeder groups. Bringing together genetic resources from various provenances into one orchard would help to maintain a high level of genetic diversity in this species.

Documentation

Information management is an important aspect of germplasm acquisition. Background information on the origins of germplasm accessions is commonly referred to as ‘passport’ data (Hummer 1999). The collection and documentation of such data are of great importance to *ex situ* conservation efforts. In 2000, a database known as the Forest Genetic Resources Information System (FORGRiS) was established under the Malaysian-German Forest Planting Material Procurement Programme (MGFPMPP). The database is modelled on the Forest Genetic Resources Information Database (FGRID) developed by the ASEAN Forest Tree Seed Centre project. It allows both centralized and decentralized management of information concerning seed and plant procurement.

The FORGRiS database holds important genecology data on approved forest reproductive materials. These include data on resource types, selected plus trees and monthly phenological monitoring, as well as seed collection, seed storage and nursery management (Thai *et al.* 1999). Initially, data for most of the commercially valuable tree species have been collected, but efforts are now being made to include also rare and endangered species. Most importantly, this system also follows the OECD scheme for certifying forest reproductive materials. To date, 47 mother trees of *N. heimii* have been selected and documented under the system in

Peninsular Malaysia, most of them in Terengganu and Perak. Many more have yet to be recorded, particularly in Pasoh Forest Reserve, Negeri Sembilan, and Balok Forest Reserve, Pahang.

Phenology

Most dipterocarp species tend to flower and fruit erratically. Such behaviour limits the supply of reproductive materials, especially seeds. It has been estimated that, in the aseasonal zones of Southeast Asia, the majority of dipterocarp species flower at intervals of 2–5 years (Wood 1956; Ashton 1969; Burgess 1972). Some species flower annually, but only a few mother trees within the population bear fruit.

Bawa (1998) reported that dipterocarps appear to be strongly cross-pollinated, a feature which could account for poor seed production if flowering trees of the same species occur infrequently and are widely dispersed. This appears to be the case in *N. heimii*, as natural regeneration beneath parent trees is rarely abundant despite annual fruiting (Elouard *et al*. 2001). Given that seeds are the only feasible method of propagating and regenerating *N. heimii*, future supplies of timber are likely to be seriously affected. Production through cuttings has not been successful for this species (A. Fauzi pers. comm.). It is important, therefore, to monitor flowering seasons and to optimize the timing of seed collection.

Several studies have been carried out to determine the ecology and biology of flowering in *N. heimii*, and to attempt to predict flowering events. Ashton *et al.* (1988) found that a fall in minimum temperatures sustained for at least 5–8 days caused flowering events eight to nine weeks later. Marzalina *et al.* (2001) analysed data from 20 years of phenological observations of *N. heimii*. They found that it tends to flower gregariously almost every year, thus confirming the observations of Medway (1972), Appanah (1979) and Yap (1985). Flowering was observed to occur either annually or biannually, and to peak between March and May, and between September and November (Figure 3). In view of the atypical flowering behaviour of *N. heimii* compared with other dipterocarps, Appanah (1979) suggested that flowering in this species is driven endogenously.

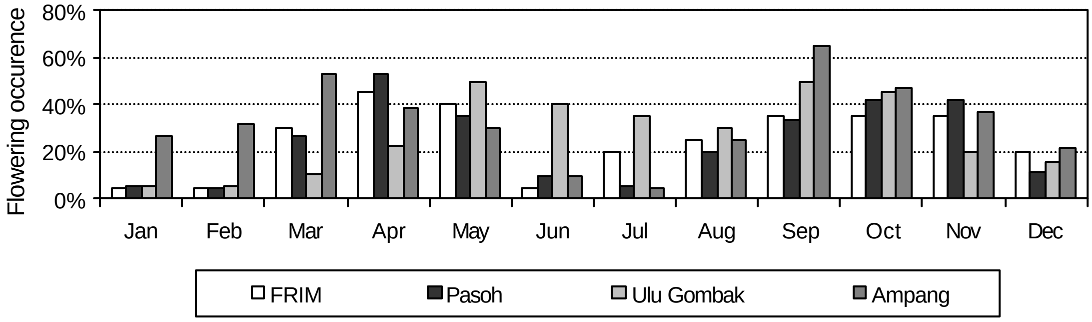

Figure 3. *Occurrence of flowering in N. heimii over a 20-year period in four forest reserves in Peninsular Malaysia. Source: Marzalina et al. (2001).*

Seed and seedling collection and storage

The timing of seed collection for *N. heimii* is critical because seeds germinate soon after falling. The seeds are wingless and heavy (5–7g), so the majority can be found under the canopy of the mother tree. The highest number of seedlings is found within 5m of the stem (Siti Rubiah 1990), and seed dispersal does not extend beyond 16m from the mother tree (Elouard *et al*. 2001). High rates of mortality near the stem could be caused by competition, insufficient light or species-specific pathogens. Levels of fruit predation are high, mainly because of insect infestations that destroy 23–37% of the seed crop before the fruits fall. Elouard *et al*. (2001) found that fungal infections, insect predation and water stress caused seedling mortality rates of 31–52%.

There is usually a high risk of losing seed viability during transit. Once collected, the seeds are best placed in the shade in an open container that circulates air readily. This will reduce heating from respiration and limit fungal growth. Marzalina *et al.* (1999) found that recalcitrant seeds are more likely to survive if they can be transported in a mobile seed-seedling chamber, which can maintain a temperature of 16–20ºC during a ten-day journey.

Germination

Seeds of *N. heimii* are highly recalcitrant. Table 1 gives moisture levels and germination rates in different freshly collected seed lots.

Table 1. *Germination and moisture content of N. heimii seed. Source: Unpublished data, Seed Technology Section, FRIM.*

Forest Reserve	Germination Rate (%)	Peak Germination Period (days)	Moisture Content (%)
FRIM	100	7–9	46.70
Ampang	100	5–7	37.78
Pasoh	95	4–9	37.69
Ulu Gombak	86	4–5	43.05
Bukit Rengit	68	4–5	50.54

Germination rates range from 68% to 100%, with moisture contents of 37.7–50.5%. Germination peaks after the fourth day of sowing. Both Sasaki and Ng (1981) and Siti Rubiah (1990) found that germination success is related to seed size and weight. Seeds over 7g in dry weight performed better in terms of growth and development than those below 5g. We also found that seed weight is positively correlated with seedling growth. Large food reserves provide an initial boost to freshly germinated seedlings, allowing the development of large root masses essential for adequate nutrient acquisition (Whitmore 1984; Foster 1986). Siti Rubiah (1990) also found that germination was highest under a light intensity of 25%, whereas seedlings grown in full sunlight grew poorly in height and biomass. These findings indicate that *N. heimii* is shade tolerant and well adapted to a closed-canopy environment.

Seed storage

A low temperature and low moisture content are fundamental conditions for long-term seed storage of most plants. Storage studies of *N. heimii* seeds have been limited by the low number of seeds available for experimentation. The conventional approach to securing a limited quantity of germplasm is immediate sowing of short-lived recalcitrant seeds in the nursery. This practice is crucial because delays in sowing are known to reduce germination rate, increase the number of abnormal seedlings and lower field emergence (Krishnapillay & Tompsett 1998).

The optimal storage period for *N. heimii* seeds is no more than 57 days at 14ºC. This yields a germination rate of 80% for seeds that have been treated with a fungicide dressing of 0.2% Benlate before storage (Yap 1981). Seeds do not die if their moisture content is reduced by half (to 28.0% from an initial 46.7%) before storage. Fungicide treatment followed by partial desiccation and storage at low temperature has also yielded good results for mid-term storage of *N. heimii* seeds.

Seedling storage

Marzalina *et al.* (1994) tested the use of a seedling chamber to slow down germination and growth of seedlings. Using this procedure, the Seed Technology Section of FRIM carried out an experiment with freshly collected *N. heimii* seeds. The surface of the seeds was treated with 0.1% Benlate/Thiram fungicide and allowed to germinate under ambient conditions in containers with moist vermiculite. Once the seeds had germinated, these containers were put into a specially built chamber where the temperature was maintained at 16ºC, relative humidity at 80% and the photoperiod at four hours a day. Germinated seeds in this chamber developed slowly, barely reaching a height of 20–25cm. After 12 months of storage, four-fifths of the seedlings were still alive. These were planted into polybags and weaned under progressively brighter conditions (from 75% to 25% shade) for two months. Post-storage survival was 70%. The hardening of seedlings is this manner is necessary before field planting.

Cryopreservation

Another *ex situ* conservation technique is cryopreservation, which is designed for long-term storage. Using this technique, recalcitrant seeds from more than ten tropical tree species have been stored in a liquid nitrogen phase (Marzalina & Krishnapillay 1999). When whole *N. heimii* seeds were desiccated to half of their initial moisture content and plunged directly into liquid nitrogen, none of the seeds survived. Most successful cryopreservation techniques have used excised embryos rather than whole seeds. Embryos are used because they are small, more resistant to desiccation and relatively uniform in size and moisture content. Table 2 lists moisture contents for different parts of *N. heimii* seeds.

Table 2. *Moisture content of N. heimii seeds. Source: Jayanthi and Krishnapillay (2000).*

	Whole Seed	Seed Coat	Cotyledons	Embryo
Initial moisture content	52.87%	31.53%	55.10%	70.70%

These figures indicate that the moisture content of the embryo is higher than other parts of *N. heimii* seed. Marzalina and Krishnapillay (1999) found that desiccated embryos of recalcitrant species, despite their higher moisture content, were able to recover after cryopreservation. We found, however, that after six hours of desiccation, the moisture content of *N. heimii* embryos fell to 27% and they were unable to survive cryopreservation. Even when the embryos were subjected to vitrification using a cocktail of 30% dimethyl sulfoxide, 15% ethylene glycol and 15% glycerol before being plunged into liquid nitrogen, their survival rate was less than 5%. More work is needed to determine the optimal combination of vitrification chemicals to prevent ice nucleation. The Seed Technology Section of FRIM has begun to produce artificial seeds based on somatic embryos. Newly emerged shoot tips from germinated seeds are being used as the source of somatic embryos. This work is still at an early stage and has yet to produce any results. However, cryopreservation techniques for the long-term storage of *N. heimii* may be available in the near future.

Conclusions

Important information for conserving *N. heimii* has been collected and documented. In recent years, basic information covering the distribution, ecology, biology, reproductive behaviour, flowering, genetic resources, growth and development, propagation and planting of this species has been updated. More research is needed, however, to sustain effective conservation programmes for this species. This research should address:

- The mating and dispersal systems of pollen and seed dispersal vectors.
- The minimum population size required for *in situ* conservation.
- The maintenance of linkages and corridors between fragmented forest areas to sustain population viability.
- Methods to increase seedling production, either through cuttings, artificial seeds or tissue culture.
- Protocols for long-term storage.
- Establishment of seed orchards.

The importance of *in situ* conservation notwithstanding, efforts to improve *ex situ* conservation techniques should not be dismissed. Using the latest developments in biotechnology, the conservation of *N. heimii* can be enhanced. All of the available techniques should be evaluated, but other options must also be considered. The need for adequate training and resources should also be addressed. Such efforts should not only concern conservationists, but also government agencies, politicians and international bodies.

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