CHAPTER 6

CONCLUSION

Despite the importance of Southeast Asia as one the largest rain forest regions, the Asian tropics have been subjected to relatively few studies on phylogeography. By using *Neobalanocarpus heimii* as a model species, this study was initiated to reveal the evolutionary history of the species in Peninsular Malaysia, including the potential glacial refugia and their post-glacial recolonization routes. *Neobalanocarpus heimii,* or locally known as chengal, is endemic and widely distributed in Peninsular Malaysia. The species produces a naturally, highly durable wood and is among the strongest timbers in the world. Owing to the high demand for its valuable timber, this species is subjected to illegal logging and might become endangered in the near future.

Fifteen haplotypes were identified from 10 intraspecific variable sites of five non-coding chloroplast DNA (cpDNA) regions: *trn*L intron, *trn*S-*trn*G spacer, *trn*G intron, *trn*K intron and *psb*K-*trn*S spacer. Based on the coalescent theory, the interior haplotypes h1, h2 and h3 can be inferred as the ancestral haplotypes (Figure 3.5). They were in the central part of a network with multiple connections, exhibited higher frequencies than tip haplotypes, and showed broader geographical distributions. Two major genealogical cpDNA lineages of *N. heimii* were elucidated in this study: a widespread southern region and a northern region. The pronounced divergence could be attributed to the long-term isolation of populations within geographically separate refugia, in which a combination of genetic drift and new mutations might be responsible for the differentiation and apportionment of the *N. heimii* genepool in the northern and southern regions.

This study also revealed the persistence of *N. heimii*'s glacial refugia in Peninsular Malaysia during Pleistocence climatic oscillations. Although some reports suggested that rain forests might disappear almost entirely from Peninsular Malaysia (Verstappen,

1997; Morley, 2000; Thomas, 2000; Brandon-Jones, 2001), small rain forest refugia are still believed to persist along the coastal regions (Corner, 1978, Emmel and Curray, 1982, Kershaw *et al*., 2001 and Quek *et al*., 2007). Results from the present study clearly revealed that the species survives in multiple refugia throughout Peninsular Malaysia (Figure 3.6a): the northwestern region (R1: Sungkop), the northeastern region (R2: Gunung Basur) and the southern region (R3: Panti compartment 16). These putative glacial refugia exhibited a higher level of genetic diversity, while the post-glacial regions were found to have a reduced level of genetic diversity with large geographic areas dominated by a single haplotype.

The putative recolonization routes for *N. heimii* throughout Peninsular Malaysia were inferred based on the level of genetic diversity, distribution pattern of haplotypes and restricted dispersal of the species. Recolonization of refugia R1 and R2 could have first expanded into the northern region of Peninsular Malaysia during the interglacial retreat, and stopped at the central regions of Pahang, Terengganu, and Perak (Figure 3.6b). These stocks might have migrated both northeastwardly and northwestwardly after the climatic amelioration. Meanwhile, recolonization of *N. heimii* throughout the southern region of Peninsular Malaysia could have commenced from refugia R3, and migrated toward the northeast and northwest respectively. Though the existence of Titiwangsa mountain range could serve as a barrier between eastern and western populations of *N. heimii*, the haplotypes were distributed evenly on both sides of the mountain ridge. Hence, Peninsular Malaysia was seemed to partition in north-south rather than east-west orientation. Apparently, it is unlikely for this lowland dipterocarp with limited seed dispersal to cross over the mountain range. Therefore, the species could have undergone contiguous range expansion from refugia R3 in the southern flank towards the northeast and northwest regions of Peninsular Malaysia.

The populations of Tersang, Pasir Raja and Rotan Tunggal are shown to exhibit remarkably high haplotype diversity, which could be the contact zones of *N. heimii* in Peninsular Malaysia. These populations comprised both haplotypes that were endemic

to the northern and also southern regions. Specifically, the contact zones with increased genetic diversity would be achieved mostly through the redistribution of the genetic information already present among populations in refugia (Petit *et al*. 2003).

An important conclusion which may be drawn from the above account, with reference to *N. heimii*, is to consider the putative glacial refugia sites, including the northwestern region (R1: Sungkop), the northeastern region (R2: Gunung Basur), and the southern region (R3: Panti compartment 16) to be prioritized for long-term management. Besides, the contact zones of *N. heimii* (Tersang, Pasir Raja and Rotan Tunggal) are another key factor in conservation considerations, in which the maintenance of genetic diversity is critical for long-term survival of a species. In addition, further studies involving other tropical tree species are essential to provide a better understanding of the potential refugia and post-glacial recolonization routes in Peninsular Malaysia. Once the information is available for many tropical tree species, a consilience of the rain forest history will hopefully come to light. Thus, by understanding the role of long-term tectonic and climatic changes in the evolution of this tropical biota, as linked with all the potential glacial refugia between species, an assortment of recovery plans could be implemented to ensure protection and conservation of the refugia sites.

New methods to match a timber log to its population of origin would signify an important forensic component in the context of stolen log traceability for the control of illegal logging and also the approach in chain of custody developed for the certification of timber from sustainably managed forests (Lyke, 1996; Chihambakwe *et al*., 1997). The inbuilt unique properties of DNA within the timber, specifically cpDNA markers, could be used to differentiate the origin of one source of timber from another. In combating illegal logging and trade, this DNA authenticity testing would require rapid development of large comprehensive databases, detailing the distribution of genetic markers and incorporating these DNA-based techniques into the traceability systems.

A population identification database and haplotype distribution map of *N. heimii* in Peninsular Malaysia were generated for authenticity testing based on four cpDNA markers: *trn*L intron, *trn*G intron, *trn*K intron and *psb*K-*trn*S spacer. Twenty-one haplotypes were identified from 10 significant intraspecific variable sites. Results clearly revealed that only the northern and southern regions of Peninsular Malaysia were distinguishable. Thus, this database could only be used to determine the wood lot of unknown origin at the regional level. Statistical procedure based on the composition of wood lot (presence/absence of haplotypes) was used to test whether a suspected timber conforms to a given regional origin. Overall, the observed types I and II errors of the database showed good concordance with the predicted 5% threshold, which might indicate that the database is useful to reveal provenance and establish conformity of wood lot from the northern and southern regions of Peninsular Malaysia.

In terms of application, this database could be applied to traceability in two different circumstances: (1) to verify the provenance of a wood lot in the context of forest certification and chain of custody certification and (2) to identify the potential population of origin of the suspected illegal harvested wood lot. The first circumstance would provide an answer as to whether a wood lot is conformed to a presumed population, whereas the second circumstance would postulate a potential population that the questioned wood lot is belonging to. As a whole, for both circumstances, the database would be primarily used to conform or reveal either the wood lot originated from the northern or southern provenance. Once the potential source region is determined, by using the individual identification profiling database that was previously developed (Tnah *et al*., 2010), an assignment test could be conducted based on multilocus genotypes of STR to determine the source of suspected timber until the level of specific population or forest reserve. The assignment test would provide the probability of the questioned wood lot belonging to a population, thus permitting a link to allocate the wood lot to its potential population of origin.

Wood can be a good source of DNA for various applications in forensic forestry and timber trade if high quality DNA can be retrieved via wood tissue extraction. For the moment though, population and individual identification databases have been established for *N. heimii* to be served as DNA authenticity tool for forensic forestry in Peninsular Malaysia (Tnah *et al*., 2009, 2010). In combating illegal logging and monitoring forest certification, a highly polymorphic nuclear short tandem repeat (nSTR) marker could be used to generate DNA profiling databases for individual identification, in which an illegal log could be matched to its original stump (Tnah *et al*., 2010), while chloroplast DNA (cpDNA) marker showing enough geographical structure could be used to differentiate the origin of one source of timber from another (Deguilloux *et al*., 2003; Tnah *et al*., 2009). However, the feasibility to use these DNA track-back systems relies on the possibility to extract DNA from dry wood.

In order to provide a general guideline for DNA authenticity testing established for *N. heimii*, this study was designed to evaluate the potential for extracting DNA from dry wood of *N. heimii* using the Qiagen kit, CTAB, and CTAB with PTB protocols. Overall, the efficacy of DNA extraction was higher for the cambium and sapwood than for the heartwood tissues. This might indicate that both the cambium and sapwood tissues can be a good source of DNA. In terms of tissue types, the Qiagen kit yielded higher PCR amplification rates from cambium tissues, while CTAB with PTB protocol showed higher amplification rates in the sapwood and heartwood tissues. The period of preservation could have strong effect on the total DNA retrieved from the dry wood, as the quality and quantity of DNA are likely to decrease throughout the year. In order to safeguard the intactness of the DNA, it is recommended that DNA extraction from the wood shall be carried out within six weeks after felling for logs and six months after felling for stumps.

In the present study, there is no obvious relationship between amplicon size and PCR amplification success rate. This might be due to the relatively short fragment length of nSTR region (93–281 bp) being used or it could be obscured by differential

degradation mechanism of the DNA sequences in a particular genome. The results also showed that chloroplast genome yielded higher amplification success rate compared with nuclear genome. One possible explanation is that chloroplast genomes are present in multiple copies per cell, and greatly more abundant than single-copy nuclear genomes, which leads to higher success rate in amplification. In addition, the PCR amplifications showed that both the nuclear and chloroplast regions can be retrieved from lumber that was heat-treated at 40 °C to 100 °C, although the phenomena of allelic dropout and inconsistency of genotyping were noted for some of the nuclear regions.

In short, with the establishment of population and individual identification databases, together with the DNA extraction protocols from the dry wood of *N. heimii*, this timber tracking system can serve as an important technical tool to monitor and verify the legality of a suspected timber in the context of illegal logging, forest certification and chain of custody certification. For the moment though, there is a need for population and individual identification databases to be established for premium timbers and for these to be used as benchmarks in ongoing scientific developments in the near future. To this end, a foolproof tracking system to verify timber legality is something very much in demand from the market place. However, overcoming the need for these databases and tracking system in all commonly traded tropical timbers will be an extremely challenging task.