CHAPTER 1

INTRODUCTION

Biogeography is the science that attempts to document and understand spatial patterns of biological diversity (Lomolino *et al*., 2006). During the late 1980s, since vast phylogenetically based study in biogeography had been conducted among taxa at or above the species level, phylogeography has been invoked as a new subdiscipline of biogeography (Lomolino *et al*., 2006). A particularly fascinating aspect of phylogeography is its power to explain how biota distributions across Earth have been influenced by historical events extending back millions of years (Beebee & Rowe, 2004). Phylogeographic studies generally do not extend beyond a Neogene time frame and focus mainly on a Pleistocene epoch (Riddle & Hafler, 2007). Important examples of recent phylogeographic studies include the discoveries of glacial refugia in ice-free region of Northern Hemisphere (Csaikl *et al*., 2002; Cheng *et al*., 2005; Szövényi *et al*., 2006; Gao *et al*., 2007; Ikeda & Setoguchi, 2007), and the Southern Hemisphere (Gardner *et al*., 2004; Shepherd *et al*., 2007), and post-glacial recolonization pathways of species (Fjellheim *et al*., 2006; Hoarau *et al*., 2007).

The nature of the biota of fauna and flora that eventually develops on each continent was the result of an interaction between their early history of origin and diversification with plate tectonics and climatic changes (Cox & Moore, 2005). Plate tectonics explains the origin and destruction of Earth’s plates as well as their lateral movement or drift (Lomolino *et al*., 2006). The plate tectonics not only shapes the distributional patterns of biota directly, by splitting and collision of land masses, but also changes the climatic patterns indirectly upon the land masses (Cox & Moore, 2005). In terms of climatic changes, fauna and flora are widely spread and dispersed during the warmer climates of the Early Cenozoic (Cox & Moore, 2005). However, climates had cooled somewhat by the Pleistocene, in the Late Cenozoic, when advancing glacial ice restricted species and populations into refugia, from which they expanded when the ice receded (Hoarau *et al*., 2007). The magnitude and rate of these changes had a dramatic effect on species ranges (Pearson & Dawson, 2003), causing the separation, migration and extinction of populations and accelerated the rate of evolution (Comes & Kadereit, 1998; Taberlet *et al*., 1998; Hampe *et al*., 2003).

Tropical rain forests are the tall, dense, evergreen forests that form the natural vegetation cover of the wet tropics, where the climate is always hot and the dry season is short or absent (Primack & Corlett, 2005). They form a broad belt around the equator, extending 5–10° to the north and south. Most of the land masses that currently support tropical rain forests have a common origin in the ancient southern supercontinent of Gondwana (Morley, 2000). The five largest of these rain forest regions are in the Amazon basin of South America, in the Congo River basin of Central Africa, on the everwet peninsula and islands of Southeast Asia, Madagascar and New Guinea (Primack & Corlett, 2005). Southeast Asia covers the third largest block of tropical rain forest, which includes most of the Malay Peninsula and the large islands of Borneo, Sumatra and Java (Whitemore, 1984, 1987), which are also known as “Sundaland”. Most Sundaland rain forests can be characterized as “dipterocarp forests” because they are dominated by large trees in the family Dipterocarpaceae (Primack & Corlett, 2005).

One of the greatest environmental influences shaping the modern rain forest communities has been the numerous cycles of glacial and interglacial periods during the Pleistocene epoch. During the glacial periods, rain forests are thought to have been confined into a few glacial refugia; and when perhumid conditions returned in subsequent interglacial periods, the rain forests expanded out of these refugia (Whitemore, 1998). Repeating of glacial and interglacial periods has significant effect on the genetic diversity of rain forests. It is expected that reduced geographical ranges associated with isolation into refugia will result in high genetic diversity within refugia sites and high genetic differentiation between refugia (Hewitt, 1996, 2004). On the contrary, areas outside refugia are often characterized by very low genetic diversity, but ‘melting pots’ can result if colonists originate from differentiated mix (Petit *et al*., 2003).

Compared with the temperate species, the persistence of refugia sites in Southeast Asia is not as well known because of a paucity of phylogeography study. There have been relatively few phylogeographical studies in Southeast Asia (Cannon & Manos, 2003; Bänfer *et al.*, 2006; Liao *et al*., 2007), and none within Peninsular Malaysia.

Illegal logging and trade are the causes for many economic and ecological problems both in the producer and consumer countries. Although rules and instruments have been established against illegal logging and trade, practicable control mechanisms to identify the origin of timber and wood products are still lacking (Degen, 2007). The control of the geographic origin in timber is susceptible to falsification, for which is usually involved the production of false paper work such as phytosanitary certificates, invoices and certificates of origin (Carr, 2007; Degen, 2007). Moreover, the available method to prove false declarations of timber origin is only in court (Finkeldey *et al*., 2007). Thus, the development of an independent DNA track-back system to test the proclaimed origin of timber based on non-manipulability is important in this circumstance. Chloroplast DNA markers, which are less variable but generally reveal much greater genetic structure, could be used to differentiate the origin of one source of timber from that of another. However, the power and possible spatial resolution of DNA markers to identify the geographic origin of timber depend strongly on the spatial genetic structure in the species distribution area and the number of sampled populations used to generate a reference database (Degen *et al*., 2001; Cavers *et al*., 2005; Nielsen & Kjaer, 2008). In combating illegal logging and trade, this tool requires rapid development of large comprehensive database, detailing the distribution of DNA markers and incorporating the DNA-based techniques into the traceability systems.

Although molecular markers hold great promise, it has been proven difficult to retrieve DNA from dry wood. The feasibility to use the DNA track-back systems relies on the possibility to extract DNA from dry wood. Hence, there is a prerequisite to generate a guideline to retrieve DNA from dry wood prior to implementation of the DNA track-back system. An assortment of factors that influence the quality of DNA, e.g. type of wood tissues, method of DNA extraction, period and method of preservation and treatment of wood product, are particularly important to be included in the guideline. In many attempts to extract DNA from dried and processed wood, a number of DNA extraction protocols have been modified to overcome the challenge of poor quality DNA and the presence of inhibitors (De Filippis & Magel, 1998; Dumolin-Lapègue *et al*., 1999; Deguilloux *et al*., 2002; Asif & Cannon, 2005; Rachmayanti *et al*., 2006, 2009; Yoshida *et al*., 2007; Tsumura *et al*., 2010). Indeed, there is no single extraction method or modification that can be applied to all sorts of dry and processed wood, as the biochemical composition and plant tissues of different species are expected to vary considerably (Rachmayanti *et al*., 2006). Also, as DNA degradation is inevitable in wood tissues, most DNA extraction protocols are commonly adjusted to avoid contamination with external DNA and to optimize the quality and quantity of DNA.

The present study was aimed at utilizing DNA markers for phylogeographic study and timber tracking by using *N. heimii* as a model species. *Neobalanocarpus heimii* or locally known as chengal, is endemic but widely distributed in Peninsular Malaysia. It is found in diverse localities, on low-lying flat land as well as on hills of up to 900 m (Symington, 1943). *Neobalanocarpus heimii* produces a naturally, highly durable wood and is among the strongest timbers in the world. It is used for heavy constructions, bridges, boats, buildings, and wherever strength is considered essential (Thomas, 1953). Under the IUCN Red List of Threaten Species, it was assigned under the vulnerable category due to a decline in the area of its distribution, the extent of occurrence and/or quality of habitat, and actual or potential levels of exploitation (Chua, 1998). Owing to the high demand for its valuable timber, *N. heimii* is subjected to illegal logging and this species might become endangered in the near future.

Objectives of the study

The specific objectives of the study were as follows:

I. To reveal the phylogeography and evolutionary history of *N. heimii*, including the potential refugia sites and their post-glacial recolonization routes in Peninsular Malaysia (Chapter 3);

II. To generate a population identification database and serve as a tracking and monitoring tool in the context of illegal logging, forest certification and chain of custody certification (Chapter 4);

III. To evaluate the potential of extracting DNA from dry wood and the accessibility of the extracted DNA to nuclear and chloroplast genomes, in order to provide a general guideline for DNA authenticity testing (Chapter 5).

Thesis organization

This thesis is outlined in a series of six chapters. Chapter 1 (present chapter) presents the general background information and objectives of the study. An overview of the phylogeography, geographical traceability, DNA extraction, chloroplast DNA markers and general biology of the studied species is presented in Chapter 2. Chapters 3 to 5 correspond to the objectives as mentioned above, and a review of the relevant literature is also included in each of these chapters. The concluding chapter (Chapter 6) presents a summary of the study and important conclusions drawn from the results presented in Chapters 3, 4 and 5. The following are related papers that have been published or submitted for publication in international refereed journals, the first of which has been published and is appended as Appendix.

I. Tnah, L.H., Lee, S.L., Ng, K.K.S., Tani, N., Bhassu, S. & Othman, R.Y. (2009) Geographical traceability of an important tropical timber (*Neobalanocarpus heimii*) inferred from chloroplast DNA. *Forest Ecology and Management*, **258**, 1918–1923.

II. Tnah, L.H., Lee, S.L., Ng, K.K.S., Bhassu, S. & Othman, R.Y. (2010) Phylogeographical pattern and evolutionary history of an important Peninsular Malaysia timber species, *Neobalanocarpus heimii* (Dipterocarpaceae). (Manuscript submitted to *Journal of Biogeography*)

III. Tnah, L.H., Lee, S.L., Ng, K.K.S., Bhassu, S. & Othman, R.Y. (2010) DNA extraction from dry wood of *Neobalanocarpus heimii* for forensic DNA profiling and timber tracking. (Manuscript submitted to *Journal of Tropical Forest Science*)