

Phylogenetic relationships of the Orang Asli and Iban of Malaysia based on maternal markers

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ABSTRACT. Malaysia remains as a crossroad of different cultures and peoples, and it has long been recognized that studying its population history can provide crucial insight into the prehistory of Southeast Asia as a whole. The earliest inhabitants were the Orang Asli in Peninsular Malaysia and the indigenous groups in Sabah and Sarawak. Although they were the earliest migrants in this region, these tribes are divided geographically by the South China Sea. We analyzed DNA sequences of 18 Orang Asli using mitochondrial DNA extracted from blood samples, each representing one sub-tribe, and from five Sarawakian Iban. Mitochondrial DNA was extracted from hair samples in order to examine relationships with the main ethnic groups in Malaysia. The D-loop region and cytochrome *b* genes were used as the candidate loci. Phylogenetic relationships were investigated using maximum parsimony and

neighbor joining algorithms, and each tree was subjected to bootstrap analysis with 1000 replicates. Analyses of the HVS I region showed that the Iban are not a distinct group from the Orang Asli; they form a sub-clade within the Orang Asli. Based on the cytochrome *b* gene, the Iban clustered with the Orang Asli in the same clade. We found evidence for considerable gene flow between Orang Asli and Iban. We concluded that the Orang Asli, Iban and the main ethnic groups of Malaysia are probably derived from a common ancestor. This is in agreement with a single-route migration theory, but it does not dismiss a two-route migration theory.

Key words: Orang Asli; Iban; Malay; Cytochrome *b*; D-loop

INTRODUCTION

The population of Malaysia is made up of over 70 distinct ethnic groups, some with their own internal variations and sub-groups, which add to the complex social and cultural mosaic (Hood, 2006). The earliest inhabitants of the country were the Orang Asli (aborigines), who are linked to the Hoabinhians of the Middle Stone Age in the Peninsular Malaysia, and the indigenous groups of Sabah and Sarawak on Borneo Island. Throughout the nation's history, inter-ethnic and cross-cultural interaction has resulted in acculturation, assimilation and syncretization (Hood, 2006). Ethnicity in Peninsular Malaysia itself is multifarious and to some extent can only be defined in terms of religion, language, custom, and geographical place of origin (Kling, 2006). The major ethnic groups in the country are the Malays, Chinese and Indians. The Malays and other indigenous peoples of the country are termed as *Bumiputera* and they constitute approximately 61% of the total population. The *Bumiputera* is a heterogeneous group and made up of different ethnic groups such as the Malays, the Orang Asli and the indigenous peoples of Sabah and Sarawak. The populations of Sabah and Sarawak are made up of no fewer than 70 ethnic groups of which at least 50 are considered to be indigenous (Harun, 2006). The country is separated by the South China Sea into two regions, Peninsular Malaysia and Borneo (Sabah and Sarawak). Sarawak's rich cultural diversity reflects its heterogeneous and multi-ethnic people with more than 20 indigenous groups, who are collectively known as Dayak (Jawan, 2006).

The Orang Asli with a total population of 133,755 comprises 18 sub-tribes. Orang Asli is distinguished into three categories (Negrito, Senoi and Proto Malay) based on physical characteristics, linguistic affinities and cultural practices (Bellwood, 1997; JHEOA, 2002; Hood, 2006; Nicholas, 2006). Each are further sub-divided into six sub-tribes. The Orang Asli Negrito group, also known as Semang, is the smallest, constituting just over 3% of the Orang Asli population and is believed to be the earliest to arrive in Peninsular Malaysia about 25,000 years ago (Hood, 2006; Lim et al., 2010). They were separated into six sub-tribes, which are the Kintak, Kensiu, Batek, Mendrik, Jahai, and Lanoh (Nicholas, 1996). They are the present day descendants of the early Hoabinhians, who were largely nomadic foragers. However, they now live in permanent settlements in the central, northern and eastern region of Peninsular Malaysia (Nicholas, 2006). The Negrito is one of several populations of the seafarer Negritos (which includes Andaman Islanders, the Aeta in Philippines and some Papuan) who are remnants of a previously widely spread Asian population (Carey, 1976).

The Senoi, which include the Mah Meri, Semok Beri, Temiar, Che Wong, Jah Hut, and Semai, is the largest of the three tribes of Orang Asli, constituting about 54% of the population. They reached Peninsular Malaysia during the second wave of migration about 8000 years ago from South Asia, the mountain areas of Cambodia, Vietnam and Burma (Nicholas, 1996; Baer, 1999). The Senoi speak Austro-Asiatic languages of the Mon-Khmer sub-group, which reflects their ancient connection with the mainland Southeast Asia (Nicholas, 1996, 2000). However, some believe the Senoi are descendants of Australoid from Australia and Veddoid from South India (Fix, 1995). The Proto Malays make up about 43% of the Orang Asli population and consist of the Jakun, Temuan, Semelai, Kuala, Kanak, and Seletar (JHEOA, 2002). They live mainly in the southern part of Peninsular Malaysia and are known to be similar to the Deutero-Malays not only from a morphological standpoint but also culturally and linguistically (Kasimin, 1991; Lim et al., 2010).

Sarawak's diverse ethnic population of over two million people comprises over 20 indigenous groups. The main native peoples, the Iban, are also known as the Sea Dayak (Jawan, 2006). Their social structures were organized around the efficient cultivation of hill rice, which was crucial for the community's survival in the past. The Iban society can be divided into three levels: the nuclear family, the longhouse community and the territorial tribe (Kedit, 2006). These organizational structures are based on spatial divisions and social inter-relationships among group members. The Iban were said to have migrated to Sarawak from outside and surrounding Borneo, causing differences in their physical features and lifestyles (Wright et al., 1971).

Various theories were proposed on the migrations of the Orang Asli into Peninsular Malaysia and the Iban to Borneo Island. However, to date, studies of the origins, the migration history as well as relationships among the Orang Asli and Bumiputera of Sarawak are limited (Lian, 2001; Macaulay et al., 2005; Hill et al., 2006; Ang, 2009; Lim et al., 2010) while most existing records are from an anthropological and historical point of view (Wright et al., 1971; Kasimin, 1991; Nicholas, 1996). At the end of the last century, specifically the previous three decades, molecular studies became widely used to study human migration and population relationships (Lutz et al., 1998; Al-Zahery et al., 2003; Endicott, 2003; Kashyap et al., 2003; HUGO Pan-Asian SNP Consortium et al., 2009; Tee et al., 2010). Mitochondrial DNA (mtDNA) analysis is particularly well suited for systematic studies of potentially closely related individuals due to the high molecular rate of evolution and maternal mode of inheritance (Md-Zain et al., 2010a,b; Rosli et al., 2011; Vun et al., 2011). Indeed, human mtDNA variation analysis has proved to be a powerful tool for reconstructing ancient migration (Torrioni et al., 1993) and detecting the origin and subsequent radiation of aborigines within the Old World and New World (Vigilant et al., 1991). In this study, the non-coding region from the HVS I gene and the cytochrome *b* (Cyt *b*) genes were used as candidate loci. Thus, the main objective of this research was to study the phylogenetic relationships among the Orang Asli in Peninsular Malaysia and the Iban from Sarawak.

MATERIAL AND METHODS

Subjects

In this study, all subjects were carefully selected by establishing their ancestry to at least three generations of Orang Asli without mixed marriages. The Iban and Orang Asli were

classified as ingroups while the Malay, Chinese, Indian, and African samples were used as the outgroup for analysis. Blood samples from each sub-tribe of Orang Asli were collected with the help of the JHEOA (Department of Orang Asli Affairs) and the District Health Clinic (Table 1). Subjects of the research comprised of male and female samples above the age of 18 years. Hair samples were collected from the Iban. The origins and ethnicity of each individual was confirmed via questionnaire. Genomic DNA was extracted from the blood samples using the phenol/chloroform method (Hillis et al., 1996; Sambrook and Russell, 2001) while DNA from the hair samples was extracted using DNeasy® Blood and Tissue Kit.

Table 1. Samples of Orang Asli and Iban used in this study.

| Tribe | Subtribe or code | Locality |
|--------------|------------------|---------------------------------|
| Melayu Proto | Seletar | Kg. Sg. Temon, Johor Bahru |
| | Jakun | Kota Tinggi, Johor |
| | Kanak | Kg. Selangi, Kota Tinggi, Johor |
| | Semelai | Pos Iskandar, Pahang |
| | Temuan | Bukit Manchung, Selangor |
| Senoi | Orang Laut | Kg. Layau, Johor |
| | Jah Hut | Temerloh, Pahang |
| | Semok Beri | Sg. Emas, Pahang |
| | Che Wong | Lanchang, Pahang |
| | Mah Meri | Pulau Carey, Pahang |
| | Temiar | Gua Musang, Kelantan |
| Negrito | Semai | Kuala Lipis, Pahang |
| | Kensiu | Kg. Lubuk Legong, Kedah |
| | Lanoh | Kg. Air Bah, Perak |
| | Kintak | Bukit Asu, Perak |
| | Mendrik | Kuala Lah, Kelantan |
| | Batek | Pos Lebir, Kelantan |
| Iban | Jahai | Jeli, Kelantan |
| | IB3 | Limbang, Sarawak |
| | IB4 | Sri Aman, Sarawak |
| | IB7 | Sibu, Sarawak |
| | IB8 | Kuching, Sarawak |
| | IB9 | Bintulu, Sarawak |

Molecular analysis

Polymerase chain reaction (PCR) amplification for HVS I was carried out using primers from Anderson et al. (1981) and Cyt *b* from Kocher et al. (1989). For both regions, amplification was performed using 50 µL total volume per reaction with about 100 ng/µL DNA template, 20 pmol/µL primers, 3.0 mM MgCl₂, 0.2 mM dNTP mix, 20 mM Tris-Cl, in pH 8.4, 50 mM potassium chloride and 0.05 U Taq polymerase. Successful amplification products were purified before sequencing.

Data analysis

The nucleotide sequences were manually edited to check for mismatch sequences before alignment by Clustal X, Clustal W and BioEdit. All sequences from the three regions were analyzed for the variable sites using PAUP 4.0b8 (Swofford, 2002). Phylogenetic trees were constructed using PAUP version 4.0b8 using two methods of analysis, neighbor-joining (NJ) with Tajima-Nei algorithms (Tamura and Nei, 1993; Swofford, 2002) and maximum par-

simony (MP) with stepwise addition of 1000 replicates in a heuristic search (Nei and Kumar, 2000; Swofford, 2002). All trees were subjected to bootstrap analysis with 1000 replicates.

RESULTS AND DISCUSSION

HVS I region

Sequence data was obtained for 27 individuals (23 ingroups and 4 outgroups). A total of 535 characters from the HVS I region were sequenced and analyzed with 468 characters found to be constant and 32 (6%) characters parsimony-informative. The MP analysis (Figure 1) showed that the Orang Asli are closely related and were clustered together in different clades supported by high bootstrap values. The high bootstrap values showed that the HVS I evolved rapidly, which was also noted by Lim et al. (2010).

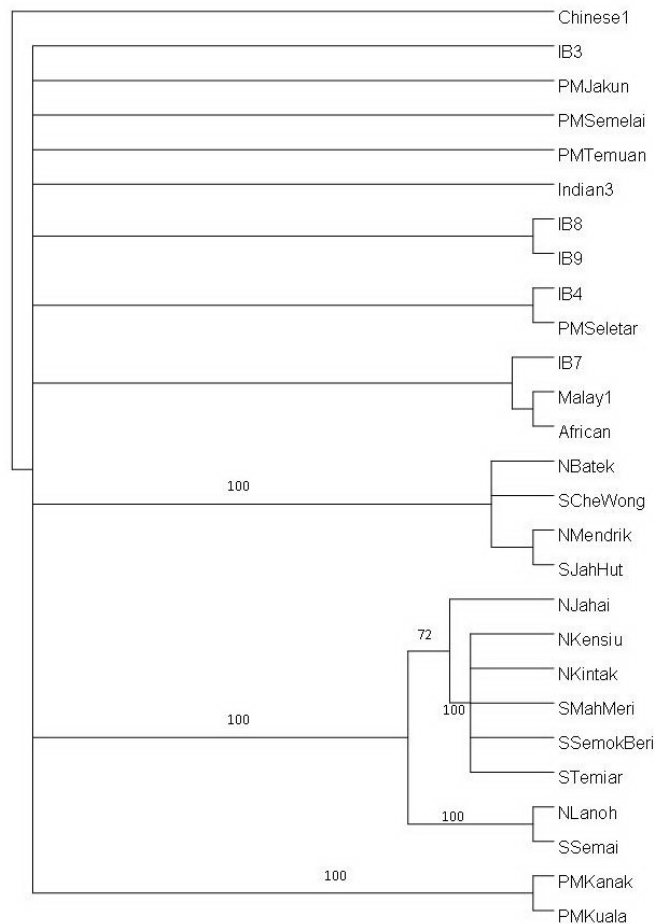


Figure 1. Maximum parsimony tree based on HVS I gene with bootstrap value on the branch.

The NJ analysis (Figure 2) of the HVS I region showed that the Iban branched the earliest from all the tribes analyzed. Samples for outgroups were constantly mixed with the ingroups and supported by high bootstrap values.

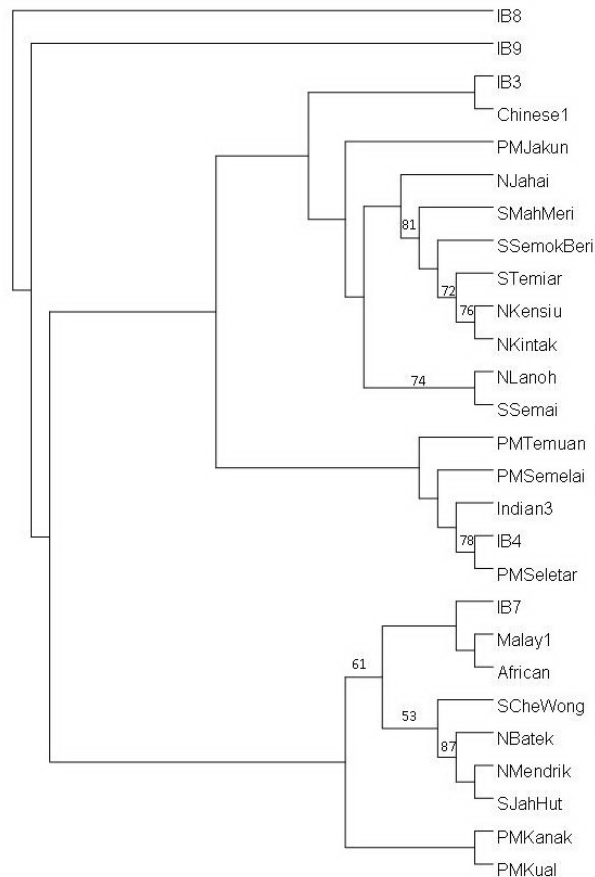


Figure 2. Neighbor-joining tree based on HVS I gene with bootstrap value on the branch.

The Negrito remains as the closest and genetically most similar tribe with the smallest genetic distance of 0.0144 (Table 2). The Iban shows the highest genetic distance at intratribal level. This may be due to the broad distribution of the Iban population across the largest State of Malaysia, Sarawak, while the Negrito settlements remain confined to the northern region of Peninsular Malaysia. Across the various tribes, the Iban and Senoi showed the highest genetic distance of 0.0269 while the Negrito and the Senoi showed the smallest genetic distance of 0.0132. The Iban consistently showed higher genetic distance as compared to the Orang Asli tribes. This is caused by the geographical location of the tribes itself where the Iban are found in Sarawak, which is separated from the Peninsular Malaysia by the South China Sea. The small genetic distances among the Orang Asli tribes showed that they are closely related to one another due to the close geographical locations of their settle-

ments, which may have been derived from a single entry into Peninsular Malaysia (HUGO Pan-Asian SNP Consortium et al., 2009).

Table 2. Genetic distances based on the HVS I and Cyt *b* genes.

| | Iban | Negrito | PMalay | Senoi | Chinese | Indian | Malay | African |
|--------------------------|--------|---------|--------|--------|---------|--------|--------|---------|
| Cyt <i>b</i> gene | | | | | | | | |
| Iban | 0.0099 | | | | | | | |
| Negrito | 0.0115 | 0.0095 | | | | | | |
| PMalay | 0.0120 | 0.0128 | 0.0106 | | | | | |
| Senoi | 0.0110 | 0.0145 | 0.0184 | 0.0137 | | | | |
| Chinese | 0.0052 | 0.0090 | 0.0102 | 0.0106 | 0 | | | |
| Indian | 0.0078 | 0.0081 | 0.0102 | 0.0106 | 0.0122 | - | | |
| Malay | 0.0078 | 0.0081 | 0.0102 | 0.0106 | 0.0074 | 0.0049 | 0 | |
| African | 0.0155 | 0.0155 | 0.0175 | 0.0178 | 0.0074 | 0.0049 | 0 | 0 |
| HVS I gene | | | | | | | | |
| Iban | 0.0291 | | | | | | | |
| Negrito | 0.0259 | 0.0144 | | | | | | |
| Senoi | 0.0269 | 0.0133 | 0.0167 | | | | | |
| PMalay | 0.0228 | 0.0198 | 0.0205 | 0.0168 | | | | |
| Chinese | 0.0281 | 0.0230 | 0.0237 | 0.0227 | 0 | | | |
| Malay | 0.0254 | 0.0224 | 0.0230 | 0.0189 | 0.0250 | 0 | | |
| Indian | 0.0219 | 0.0218 | 0.0224 | 0.0157 | 0.0230 | 0.0019 | 0 | |
| African | 0.0274 | 0.0244 | 0.0250 | 0.0209 | 0.0270 | 0.0231 | 0.0231 | 0 |

PMalay = Proto Malay.

Cyt *b*

Twenty-seven samples consisting of 23 ingroups and 4 outgroups were used in the analysis. A total of 412 characters were analyzed with 390 constant and 15 (3.6%) parsimony-informative characters. The phylogeny constructed with the Cyt *b* showed that the Asian populations are closely related while the African one was separated from it (bootstrap value of 78%, Figure 3). The Orang Asli and Iban are mixed at various clades on the phylogeny tree supported by high bootstrap values. As D-loop, the close relationships of the subjects in Cyt *b* locus also suggested a single wave of migration and entry into South East Asia (HUGO Pan-Asian SNP Consortium et al., 2009).

Based on the Cyt *b* gene, the Negrito remains as the closest tribe (0.009) while the Senoi recorded the highest genetic distance of 0.014. The Negrito remains as the smallest tribe among the Orang Asli. The Senoi are the largest tribe of the Orang Asli and their settlements, ranging from coastal to mountain regions, are widely spread across various states in central Peninsular Malaysia. The wide range of Senoi lifestyle backgrounds also contributed to their diversity as they do not closely associate with one another. The genetic distances among the ingroups ranging from the smallest of 0.0115 (Iban and Negrito) to the highest of 0.0183 (Proto Malay and Senoi) showed the populations in Malaysia to be admixed but remain related at a distinct point. The data showed considerable gene flow among the populations in these clusters.

Various genes used in the analysis help to map the phylogenetic relationships based on maternal inheritance. HVS I is one of the three most variable regions in the D-loop of mtDNA (Malyarchuk et al., 2002). Rapid evolution in the mtDNA control region also caused transitions, differences in nucleotide frequencies and high variability in the nucleotide substitution rate (Tamura and Nei, 1993). The mtDNA will help to define the relationships of the tribes at various points in history and subsequently map the migration routes.

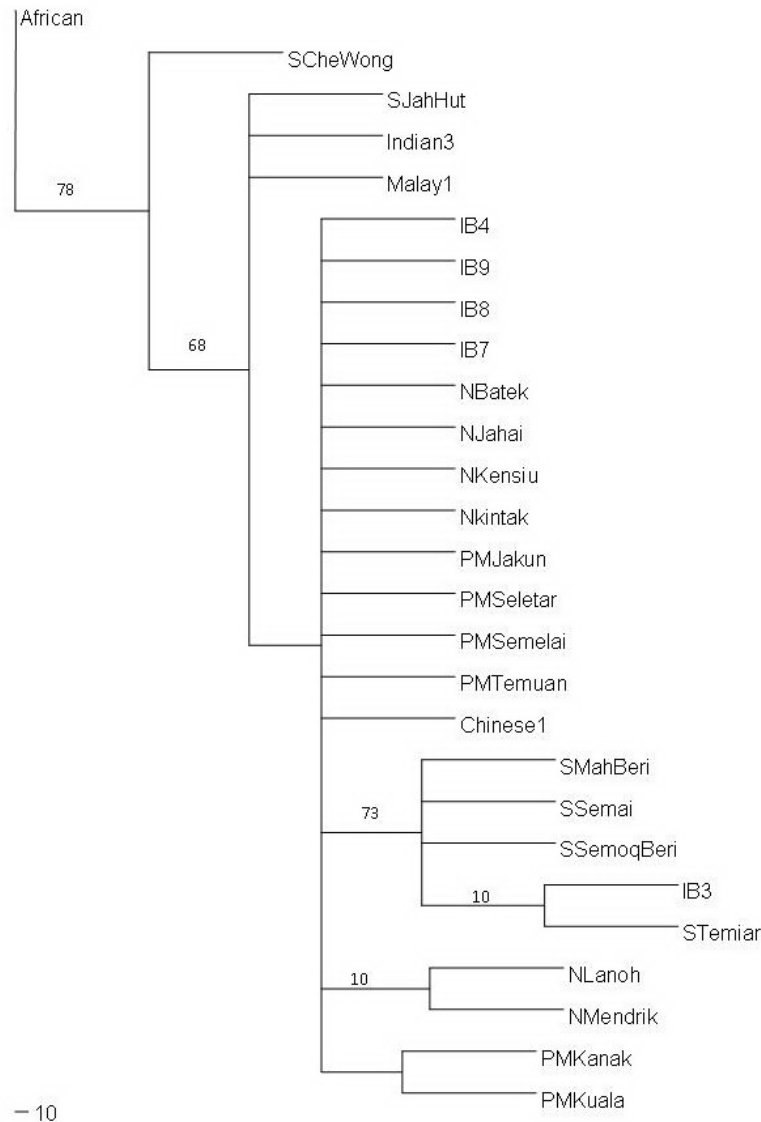


Figure 3. Neighbor-joining tree based on Cyt *b* gene with bootstrap value on the branch.

In conclusion, the mtDNA variation shows strong evidence for indigenous origins within Malaysia. The small genetic distance between the Iban from Sarawak and Orang Asli in Peninsular Malaysia suggests that they originated from a single source and represents gene flow between the tribes. Data gathered may open new insights into the dispersal and migration of the people of South East Asia. Without dismissing the theory of a two-route migration, the data pointed towards a single-route migration that supports the different waves of entry into South East Asia, and Malaysia in particular.

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