CONSERVATION GENETICS OF THE BORNEAN ELEPHANT (*Elephas maximus*) IN SABAH

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DECLARATION

I hereby declare that the material in this thesis is my own except for quotations, equations, summaries and references, which have been duly acknowledged.

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ABSTRACT

CONSERVATION GENETICS OF THE BORNEAN ELEPHANT (*Elephas maximus*) IN SABAH

The Bornean elephant was claimed to be native to Borneo since its Pleistocene migration; when the Sunda shelf was exposed allowing the migration of elephants from mainland Asia to the Bornean landmass. As the sea levels eventually raised creating Borneo as an island, this population is now isolated from other Asian elephant populations. It therefore is crucial to have an idea on its genetic diversity to ensure the best conservation and management plan on this species. Previous study showed that the Bornean elephant has low genetic diversity to be compared to other Asian elephant population and this work is meant to relook at the issue by both increasing the number of samples and expanding the sampling area through all of its known range in Borneo. From 2005 to 2007, 779 dung samples have been collected from all elephant ranges in Sabah, mainly in the Lower Kinabatangan floodplain, Tabin Wildlife Reserve, Maliau Basin, and the forest reserves of Deramakot, Ulu Segama-Malua, Kalabakan, Gunung Rara and Kuamut. DNA were extracted from 90% of the samples. Forty seven dung samples have been sequenced using the same fragment of mitochondrial DNA than was previously used in a joint study by Columbia University, Sabah Wildlife Department and WWF-Malaysia. Only one haplotype was found, which might indicate that this population was introduced to the Borneo Island. A total of 289 dung samples were genotyped for 18 nuclear markers to identify individuals and characterised the genetic diversity within and the genetic differentiation between sub-populations in Sabah. Results showed that there is a very low genetic diversity in the Bornean elephant population, with a mean number of alleles (MNA) per locus of two, a mean expected of 0.30 and a mean observed heterozygosity of 0.21. Lower Kinabatangan showed the highest genetic diversity (MNA= 3.3) and Maliau Basin has the lowest genetic diversity (MNA= 1.6). Based on the genetic results from this study and the historical events as elucidated by Cranbrook *et al.* (2008), I propose a new revision of Fernando *et al.* (2003b) which claims that the Asian elephant in Borneo is native to the island. I discuss these results and put them in a broader context of population management and conservation.

ABSTRAK

Gajah Borneo telah dikatakan wujud di Borneo setelah bermigrasi dari tanah besar Asia ke kepulauan Borneo sewaktu Pentas Sunda terdedah pada zaman Pleistocene. Setelah air laut meningkat menjadikan Borneo sebagai suatu kepulauan, populasi gajah ini telah terpencil daripada populasi gajah Asia yang lain. Oleh itu, maklumat berkaitan kepelbagaian genetik amat penting untuk diketahui bagi memastikan perancangan terbaik dalam memulihara dan mengurus spesies ini. Kajian sebelum ini menunjukkan bahawa gajah Borneo mempunyai kepelbagaian genetik yang rendah jika dibandingkan dengan populasi gajah Asia yang lain. Maka, kajian kali ini adalah untuk memastikan isu ini dengan menambahkan jumlah sampel dan memperluaskan kawasan persampelan meliputi kesemua banjaran taburan populasi gajah di Borneo. Bermula dari 2005 hingga 2007, 779 sampel tahi gajah telah dikutip daripada keseluruhan banjaran taburan populasi gajah di Sabah iaitu Lembangan Sungai Kinabatangan, Hutan Simpan Hidupan Liar Tabin, Lembangan Maliau, Hutan Simpan Deramakot, Ulu-Segama Malua, Kalabakan, Gunung Rara dan Kuamut. DNA telah diekstrak daripada 90% sampel tahi gajah yang telah dikutip dimana 47 sampel tahi telah dijujuk dengan menggunakan bahagian mitokonria DNA yang sama seperti yang telah digunakan oleh kajian sebelum ini yang telah dijalankan oleh Columbia University, Jabatan Hidupan Liar dan WWF-Malaysia. Keputusan yang diperolehi adalah sama dengan keputusan kajian sebelum ini iaitu gajah Borneo mempunyai jujukan mitokonria yang unik bagi gajah Asia. Manakala, dengan menggunakan 18 penanda DNA nukleus, sebanyak 289 sampel tahi gajah telah digunakan bagi tujuan identifikasi individu, kajian diversiti genetik bagi setiap populasi dan meneliti perbezaan kandungan genetik di antara sub-populasi gajah di Sabah. Keputusan menunjukkan bahawa gajah Borneo mempunyai aras diversiti genetik yang sangat rendah dengan purata jumlah alel adalah dua bagi setiap lokus, purata bagi heterozigositi yang dijangka adalah 0.30 dan purata bagi heterozigositi yang dicerap adalah 0.21. Populasi gajah di Lembangan Sungai Kinabatangan mempunyai diversiti genetik yang tertinggi (MNA= 3.3) manakala Lembangan Maliau mempunyai diversiti genetik yang terendah (MNA= 1.6). Berdasarkan keputusan yang diperolehi di dalam kajian ini dan kesan-kesan sejarah seperti yang diterangkan oleh Cranbrook et al. (2008), maka saya mencadangkan agar pendirian Fernando et al. (2003b) yang mengatakan bahawa gajah Asia di Pulau Borneo berasal dari pulau ini patut dikaji semula. Keputusan yang telah diperolehi telah dibincangkan yang mana turut meliputi aspek pengurusan dan pemuliharaan gajah Borneo di Sabah.

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CHAPTER 1

INTRODUCTION

The Asian elephant (*Elephas maximus*), which is historically distributed over vast parts of Asia including West Asia (Tigris-Euphrates), East Asia (Persia), the Indian sub-continent, South and Southeast Asia and the islands of Sri Lanka, Java, Sumatra and Borneo, is now being threatened as a results of the systematic destruction of their habitats (Sukumar, 2003). Today, it is distributed among 13 countries in Asia including India, Sri Lanka, Nepal, Bhutan, Bangladesh, Myammar, Thailand, Laos, Cambodia, Vietnam, China, Malaysia (Peninsular Malaysia and Sabah) and Indonesia (Sumatra and Kalimantan) (Sukumar, 2000).

Fernando *et al.* (2003b) carried out the first molecular study on the Bornean elephant and suggested that the elephant is originated from Sundaland, different genetically from all other elephant populations analyzed (including mainland Asia, Sumatra and Sri Lanka) and is native to Borneo, suggesting it to be a high priority for conservation. They had also showed that the genetic diversity of the Bornean elephant is lower than the other Asian elephant populations. Much of the similarity limited historical range on this island has been transformed into large-scale plantations and other anthropogenic activities. In view to this bizarre twist of both the unique distribution and the written history of the elephants in Borneo, I embarked into studying its genetics- a tool that could perhaps shed lights onto many questions especially on its origin and taxonomic standing. This study should also highlight the "genetic health" of the Bornean elephant.

The main objective of this study is not to answer the questions on the origin of the Bornean elephant; many fine debates and arguments have taken place (Crankbrook *et al.*, 2008; Shim, 2000; Shim, 2003). It is more to evaluate if the Bornean elephant was genetically healthy and help to find the best management practice to maintain the population. However, I tried to add more comprehensive information, following a recent publication by Cranbrook *et al.* (2008) which added valuable historical facts to the origin of the Bornean elephant. In this study, I have first increased and expanded the sampling locations and the number of samples used for mtDNA analysis. Secondly, I used 18 new microsatellite markers that have been specifically developed for the Asian elephant (Kongrit *et al.*, 2007) to look at the genetic diversity in and genetic differentiation of the Bornean elephant in Sabah.

The main concern of conservation genetics is to avoid deleterious genetic effects once populations are subjected to decreasing size and habitat fragmentation (Frankham *et al.*, 2002). A fragmented habitat can result in a population losing its genetic variability. The population might consequently face inbreeding depression (Amos and Balmford, 2001) but, a contradictory example is also available on the case of the cheetah which survives on extremely low genetic diversity (see Wiltings *et al.*, 2007). A consequence of inbreeding depression can be the inability for a species or a population to adapt to changing environment (Freeland, 2005). The knowledge gathered on the demographic history and genetic diversity of populations will show and guide the range states on how to manage and find solutions to ensure the conservation and continuous existence of many species and/or populations (Beaumont and Bruford, 1999).

The usefulness of the application of microsatellite markers in assessing the genetic characteristics of wildlife species is undeniable (Fredsted *et al.*, 2005; Goossens *et al.*, 2005; Mateus *et al.*, 2004; Wiseman, *et al.*, 2000). However, the use of microsatellite DNA markers in large mammal conservation genetics is limited by the logistic difficulties in obtaining optimal number of sources for DNA from free-ranging animals (Vidya and Sukumar, 2005). With the discovery of non-invasive methods (see Goossens and Bruford, 2009 for a review), many of these problems have been solved and scientists can now extracts DNA from many sources such as dung, shed hairs and feathers, sloughed skin and even eggshell (Goossens and Bruford, 2009). With the theoretically numerous advantages, microsatellite markers are currently among the most powerful tools in conservation genetics.

This study aimed to assess the degree of genetic diversity and variability within the Bornean elephant population and the levels of genetic differentiation between isolated populations in Sabah. Microsatellite markers amplified from DNA isolated from dung were used to assess these parameters. The previous study on genetic diversity of the Bornean elephant showed that the Bornean population has very low genetic diversity as compared to other Asian elephant populations (Fernando *et al.*, 2003b). By using more data and DNA markers that can produce a high amount of genetic information (Goossens and Bruford, 2009), this study hopes to increase our current knowledge on the Bornean elephant. To accomplish the aims of the study, the objectives are as follow:

1) To assess genetic diversity in the Bornean elephant population in Sabah and between their different ranges.

2) To see if the genetic data could explain the origin of the Bornean elephant

CHAPTER 2

LITERATURE REVIEW

2.1 Elephant of the World

Of all the Proboscidean members, only two species survived until now that is the African elephant (*Loxodonta africana*) and the Asian elephant (*Elephas maximus*). Both of this species share the same morphological, behavior and physiological characteristics with some adaptations according to it needs. For instance, the body and ear size of the African elephant is larger than the Asian elephant. The larger ear provides a significant amount of surface area for heat loss which is useful to reduce their body heat in the savanna (Shoshani, 1991). In the Asian elephant, only the males carry tusks while the females bear small tusks or most of the time these small tusks are absent (Luxmoore, 1991) while both sexes have tusk in the African elephant. The African elephants also have more toenails with four or five at the forefeet and three to five at the hind feet while the Asian elephant has five and four to five respectively (Shoshani, 1991). Another distinct physical appearance of the African elephant is that the tip of the trunk has two "fingers" while the Asian elephant has a single "finger" (Figure 2.1).

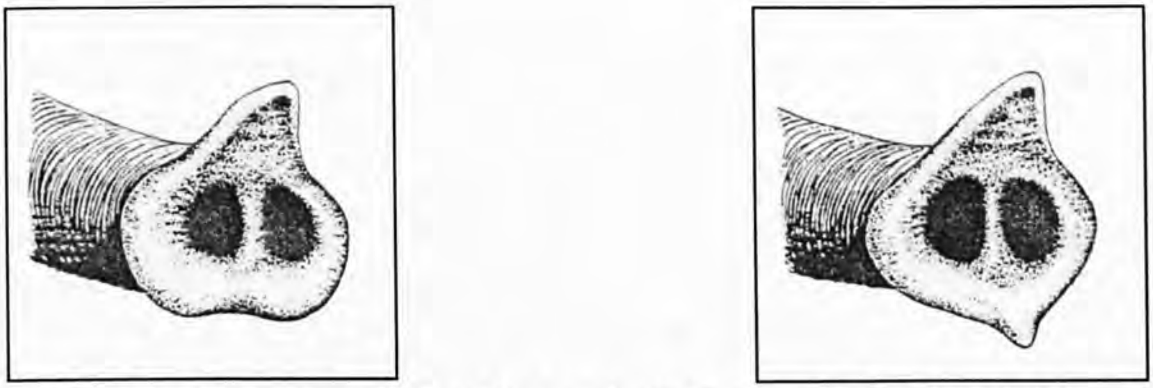

Figure 2.1: **The Asian elephant has a single "finger" tip (on the right) while the African elephant has two "fingers" tip (on the left).**

To date, two sub-species of the African elephant are identified that is the savanna elephant (*L. a. cylotis*) and the forest elephant (*L. a. africana*). While, three sub-species of the Asian elephant that is the Indian elephant (*E. m. indicus*), the Sumatra elephant (*E. m. sumatrensis*) and the Bornean elephant (*E. m. borneensis*). The sub-species status is granted base on the difference of morphological characteristics, habitat and its genetic makeup (Shoshani and Eisenberg, 1982; Roca *et al.*, 2001; Fernando *et al.*, 2003b). These two species share the same biology characteristics. The basic social unit is the family consisting of an adult cow and her immature offspring. Elephant society is multi-tiered, starting from the mother–calf (or several offspring) unit and moving on to larger joint-family units with several adult cows, the 'kin' or bond group (two to four family units that associate frequently), the clan (a number of family units and bond groups in an area that coordinate their movements), the subpopulation (a number of clans using a particular part of the range) and the whole population occurs once a year (Gobush and Wasser, 2009). When a bull is in musth the testosterone in the blood increases to high levels, the temporal glands secrete a pungent fluid, the animal shows heightened aggression towards other bulls and it has a better chance of successful mating with an oestrous female (Hollister-Smith *et al.*, 2007).

2.2 A Brief History of the Asian Elephant

Early distribution of the Asian elephant was ranged from the Tigris-Euphrates through Persia and Indian sub-continent towards Southeast Asia. However, the current distribution of the species is restricted to fragmented forests of the Indian sub-continent (India, Nepal, Bhutan and Bangladesh), continental Southeast Asia (China, Burma, Thailand, Cambodia, Laos, Vietnam and Malaysia) and islands (Sri Lanka, Sumatra and Borneo) (Sukumar, 1989).

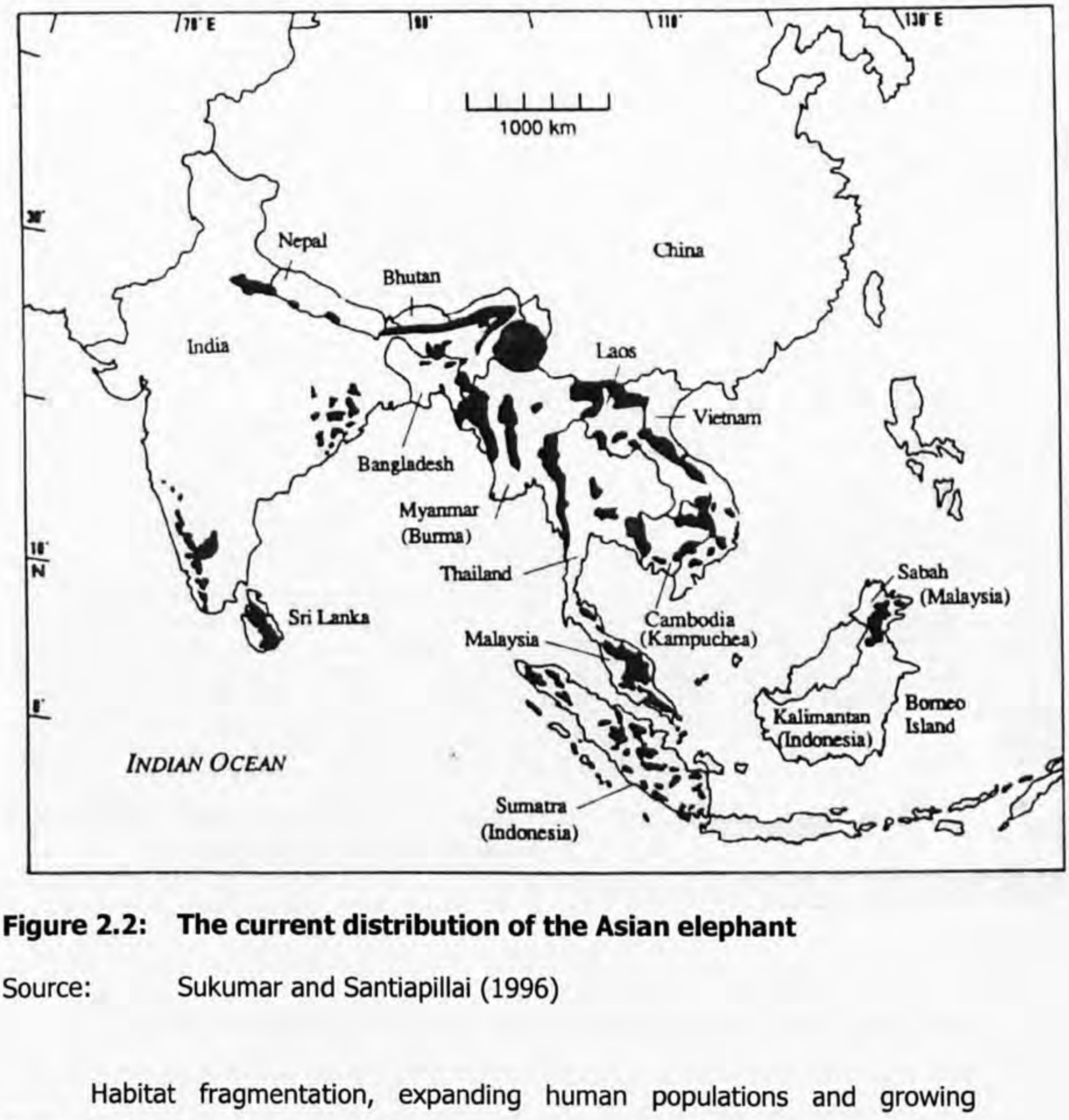

Figure 2.2: The current distribution of the Asian elephant

Source: Sukumar and Santiapillai (1996)

Habitat fragmentation, expanding human populations and growing demands for natural resources have a major impact on the conservation of the Asian elephant (Leimgruber *et al.*, 2003). In the early 1980s, the population of the Asian elephant was estimated to be more than 200,000 individuals (Shoshani

Sukumar *et al.* (1998) have found out that in one of the elephant populations in India; most of the elephants that have been killed for their tusks were between 10 to 20 years old. It is proven that poaching activities for tusks can extremely skew sex ratios in elephant populations (Sukumar *et al.*, 1998; Barnes, 1999)

In Sabah, habitat loss, HEC, poaching and logging are among the problems identified by Ambu *et al.* (2000) that might jeopardize the survival of the Asian elephant in the state. The rapid conversion of land to oil palm plantations contributed to the habitat fragmentation cutting off connections between populations. The situation is worsened as logging activities provide an easy access for poachers. Although poaching has never been a serious problem for elephants in Sabah, there were several cases where elephants were killed following their intrusion into oil palm estates (Ambu *et al.*, 2000). In the Lower Kinabatangan Wildlife Sanctuary (LKWS), the elephants are not only going into oil palm plantations but they also destroy orchards and cemeteries, which leaves a negative sentiment on the villagers.

Table 2.1: Major conservation issues of Asian elephants in each Asian country.

Country	Asian elephant Conservation Problems
Bangladesh	- Conversion of forest to other land uses - Poor financial status to manage reserve forest - Human-elephant conflict.
Bhutan	- Inadequate trained personnel to manage protected area - Low level of scientific expertise
Myammar	- Political situation - Inadequate conservation area. - Poaching and crop raiding
Cambodia	- War - Inadequate trained personnel to manage protected area
China	- Deforestation and hunting - Lack of law enforcement
India	- Poaching - Elephant captures for domestication - Mortality due to outbreaks of epidemic diseases and drought - Conversion of forests to other land uses
Indonesia	- Logging - Conversion of forests to other land uses - Habitat fragmentation
Malaysia	- Logging - Conversion of forests to other land uses - Habitat lost
Nepal	- Cultivation - Clearing forests
Sri Lanka	- Habitat loss and forest clearance
Thailand	- High density but insufficient areas - Conversion of forests to other land uses
Laos	- War - Ineffective control on hunting - Has no protected areas.

Source: Santiapillai and Jackson (1990)

2.3 Elephants and Genetics

The endless series of habitat loss for agricultural expansion, logging activities and human settlement means that the chance of different elephant populations to meet is restricted and this situation will interfere the gene flow between populations, which in turn will cause inbreeding in the populations (Fickel *et al.*, 2007). Inbreeding occurs when closely related individuals mate among themselves, increasing the number of homozygote individuals and resulting in the loss of heterozygosity (DeYoung and Honeycutt, 2005; Freeland, 2005). Heterozygosity or genetic diversity is important for a population to adapt with changing environment such as weather fluctuations (*e.g.* El Niño), parasitism, population bottleneck and environmental stress (food and water shortage) (Amos and Bradford, 2001; Keller and Weller, 2002). The effect of inbreeding depression has been observed in many other animal species which gave a significant reduction in survival, reproductive success, hatch rate, population growth and population dynamic (Keller and Weller, 2002; Keller *et al.*, 2007; McGowan *et al.*, 2007). Small and isolated animal populations are also threatened by the loss of genetic variation when alleles are randomly fixed and due to genetic drifts (Keller and Waller, 2002).

With the great improvement from all aspects of the molecular tools, techniques and genetic data analysis, molecular techniques has become a routine procedure used in the conservation efforts of an animal species (Haig, 1998; Excoffier and Heckel, 2006). Molecular tools provide valuable data on diversity through their ability to detect variations at the DNA level (Freeland, 2005). Molecular techniques can be used to define taxonomic status, hybridization, genetic diversity, identification of population structure and identification of unknown individuals (Haig, 1998). Therefore, it comes as no surprise that from the last 20 years, many studies on the genetics of the Asian elephant has revealed some interesting findings and it has lifted our knowledge on this species (Fernando *et al.*, 2000; Vidya *et al.*, 2005).

Good and reliable data on population sizes and distribution is generally lacking for most countries. The current Asian elephant population is "estimated" to be between 15,000 to 31,600 elephants, spread across the 13 range States; however this figure has been quoted for decades (Table 2.2). If compared to the African species, which is estimated to have 600,000 animals, the number of Asian elephants is evidently much lower (Levine, 1998). Like the African forest elephant (*Loxodonta africana cyclotis*), the major factors that hinder the effort of studies to be performed on the wild Asian elephant is the limited access and visibility in the habitat that the elephant lives in. Of all the Asian elephant subspecies, the Indian elephant is the most well studied population. From the literature published on the Indian elephant, the studies include the status and distribution, home range, feeding behavior, human-elephant conflicts (HEC) and genetics (Daniel and Datye, 1993).

Table 2.2: The minimum and maximum number of the Asian elephant.

Country	Numbers	
	Minimum	Maximum
Bangladesh	200	350
Bhutan	60	150
Burma	3,000	10,000
Cambodia	2,000	2,000
China	150	300
Indonesia		
Kalimantan	100	500
Sumatra	2,800	5,000
Laos	2,000	3,000
Malaysia		
Peninsular Malaysia	800	1,000
Sabah	500	2,000
Nepal	50	90
Sri Lanka	2,700	3,200
Thailand	1,300	2,000
Vietnam	1,500	2,000
TOTAL	**17,160**	**31,590**

Source: Sukumar (1989)

2.4 The Bornean Elephant

The Bornean elephant is found living in the east coast of Sabah and in a small area of the northeast of Kalimantan (Figure 2.4) (Santiapillai and Jackson, 1990). Figure 2.4 shows the past (locations covered with yellow color) and present (locations covered with orange color) distribution of the Bornean elephant in Sabah. Once again, the question of why this animal is only confined to a small and very localized region of the Borneo Island is yet to be determined but it is believed that its habitats are restricted to the region with most fertile soils and abundant natural resources and the lowest hunting pressure (Payne *et al.*, 1985). However, this observation is debatable as salt licks and most minerals can also be found along west coast of Sabah (Shim, 2000).

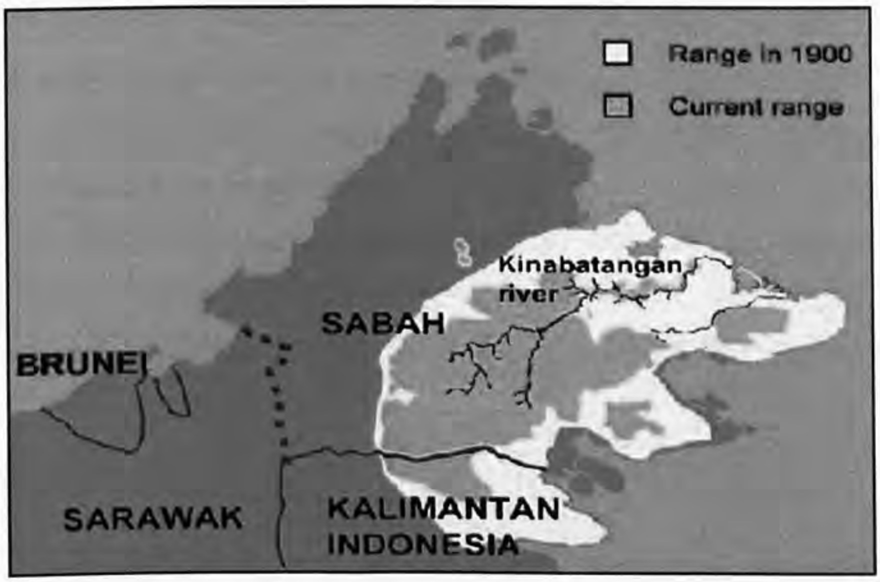

Figure 2.4: The Bornean elephant present distribution in Borneo Island.

Source: Fernando *et al.* (2003b)

In 1982, it was estimated that there were 500 to 2,000 elephants based on the location of known herds (Davies and Payne, 1982), but the latest estimation by showed that Sabah has a minimum of 1,100 and a maximum of 1,600 elephants (Ambu *et al.*, 2000). The most recent estimation by Ambu *et al.*

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