Chapter 7: Discussions and Conclusions

7.1 Introduction

To the best of my knowledge, this is the first study that combines Biotope Maps, Radio-Tracking Telemetry and Molecular Genetics to better understand the ranging patterns of the Slow Loris *Nycticebus c. coucang,* and to document the spatial interactions among individuals when inferring relatedness of individuals sampled in the wild, as well as in captivity.

The study presented here processed field radio-telemetry GPS locations into a movement documentation of the *Nycticebus c. coucang* from which I demonstrated frequently-used Biotopes and Movement Traveling Corridors. The study provides important and novel information regarding combination of methodologies, structures of traveling corridor, daily distances, used core areas of individuals and the implication of inferring relatedness of the *Nycticebus c. coucang* in the wild.

7.2 Biotope Mapping and Analysis

Chapter 4 presented biotope classification maps for the Bukit Boloh and Cempaka study areas in the District of Temerloh, State of Pahang. Overall, there is a high degree of confidence in the classified results, a fact based largely on the visual ground-truthing assessment made on several occasions during the field work. It is noteworthy to mention that the study areas included large homogenous patches, for example, continuous forest and agricultural fields which are easily mapped. The level of accuracy sought and obtained when classifying the biotopes of the study areas using satellite imagery and digital lot (cadastre) maps can be arbitrary and measures depend on the classification employed, the scale

of the area considered in the study area, as well as the spatial resolution of the satellite images and the digital lot map used in the analysis. Some factors that could influence the accuracy of the classification are the type and quality of input data, these being GPS, GIS, Digital Lot Maps and Satellite Imagery data (Michael, 2003; Ling, 2010).

7.3 Ranging Patterns

7.3.1 Forest Vertical Use

The movement heights reported by Wiens (2002) in his Manjong study area in the State of Perak, were between 1.8 to 35.0 m, with no preferential height reported. In the present study the vertical of use of the forest by *Nycticebus c. coucang* showed a greater preference for heights less than 15 m, and a least preference for heights over 20 m. Other studies carried out in diurnal solitary primates such as Golden-faced Sakis reported that these species moved in lower strata of the forest (Fleagle, 1980; Setz, 1993; Mittermeier van Roosmalen, 1981). Interestingly, these primate species are cryptic to a certain extent as is the *Nycticebus c. coucang*. This preferential height might be related to adaptive anti-predatory behavior for these solitary and shy primates since they are less able to detect the presence of terrestrial predators and preferred to use of height from 8 to 15 m (Vie, 2001).

7.3.2 Sleeping Sites

The sleep patterns of the *Nycticebus c. coucang* found in the present study were generally consistent with regard to the different sleeping sites (trees, shrubs, and palms) as reported by Wiens (2002). However, the percentage of recorded occurrence was higher in the present study. It was suggested by Anderson (1984) that primate groups that defend territories, containing only one male, have

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fewer sleeping sites, while multi-male groups accumulate more sleeping sites. In the present study there was present only one male per group (with the exception of group E in my study). However, the number of sleeping sites identified was more when compared with previous studies. The explanation might be that sleeping sites are near to human settlement surrounded by forest, and these then would provide the *Nycticebus c. coucang* good protection from predators (Blaffer, 1977). Another possible factor influencing the number of sleeping sites obtained in this study could have been the abundance of suitable trees since these allowed the *Nycticebus c. coucang* to choose among many sites (Aldrich-Blake, 1970). Furthermore, by having several sleeping sites *Nycticebus c. coucang* did not have to make a long return journey to the day-before sleeping site, especially when it has been proved that these individuals moved through inter-patch forest. Nevertheless, the adult female in this study, Bonita, did go back to the same sleep site, spending time and energetic costs of travelling (Chapman, 1989), most likely because her offspring was too young to be left alone for few days as was also seen in the case of two other infants in the study, Little and Adopt.

Primates sharing a sleeping site with one or more neighbour groups of conspecifics on the same night have been reported by several authors (Altman, 1970; MacDonald, 1982). Wiens (2002) reported that individuals *Nycticebus c .coucang* were seen sleeping in threesomes. I did not observe more than two individuals on the same sleeping tree on the same night during the entire study period. Moreover, neighbouring groups of *Nycticebus c. coucang* did not share any sleeping site during the study period. I did, however, observe two adult males (which were related individuals) from the same group sleeping on the same tree and in physical contact. It has been proposed that sympatric primates may share the sleeping areas, owing to the chance meeting of two species when near to food source, or on a travel route and sleeping sites can usually be

contiguous with some overlap of the two species at night, as reported in studies of the Orangutans and Long-tailed Macaques (Fittinghoff & Lindburg, 1980).

Sympatric living in animals may induce some form of interactions. Baldwin and Baldwin (1973) reported that Squirrel Monkeys and Howler Monkeys (*Alonotta villosa*) respond reciprocally to each other's alarm calls, thus benefitting from these signals. This study showed evidence of sympatric living among the nocturnal *Nycticebus c. coucang* with two other diurnal primate species (*Macaca fascicularis* and *Presbytis femolaris*) with which they shared the same sleeping sites. This researcher observed no discernible aggression displayed by these diurnal species towards the *Nycticebus c. coucang* at the time of sun-rise when they, the former, started with their own daily activities.

There was no obvious predation pressure for the *Nycticebus c. coucang* population within the Bukit Boloh and Cempaka areas, although human impact in the form of settlement pressure and the use of land for agriculture purposes was evident. Predation by mammals such cats or snakes, which had been reported by Wiens (2002) appeared to be virtually nonexistent in my study areas.

For sleeping sites, the *Nycticebus c. coucang* showed preferences for middle trees with thick foliage, and for avoiding rubber plantations. Tree species such as *Mariantree* and *Silverback* were chosen as sleeping sites, owing to their bushy crowns, and that these were located in dense tangles of vegetation.

It has been observed that, normally, primates choose to sleep in trees which have no low branches (Anderson & McGrew, 1983). A common feature of the sleeping places is that they are difficult, if not impossible, for potential predators to reach or to scale up the trees (Hall, 1965). Some groups of primates are reported to prefer tall sleeping trees, for example the *Colobus ubyssinicus* as

reported by Haddow, (1952); the *Papio ursinus (*Hamilton, 1982); the Bonnet Monkeys (*Macucu rudiutu)* (Koyama, 1973); and the *Macaca fascicularis,* (Kurland, 1973). However, smaller species such as Geoffroy's Tamarins (*Saguinus oedipus*) are reported to chose sleeping sites below 7 m (Dawson, 1979); Marmosets (*Callithrin humeralifer*) selected 5 - 6 m heights (Rylands, 1981); and the Pygmy Marmoset preferred 7 to 10 m tree heights (Soini, 1982).

In this study, the *Nycticebus c.coucang* followed the same height characteristics of these smaller species, as mentioned above. They often slept among medium or large bushy trees/palms and shrubs of between 5 to 20 m in height, but occasionally were found to be sleeping in small shrubs near the orchards cover by foliage shrubs as low as 3 m in height. In Bukit Boloh Belukar trees of 3 - 4m heights, and covered with spiny rattan palm, were consistently used by individuals for their sleeping sites. This study partially corroborates what was reported by Jolly (1972) where he stated that smaller species use concealment among dense vegetation to avoid predators.

Several authors have described the possible selection of sleeping sites in relation to weather conditions (Kummer, 1981; Anderson, 1982). Dayong (2010) and Ren (2009) reported that the Snub-Nosed Monkey slept for a longer night-time period during winter, and the shortest period in summer, and that poor weather also influenced their sleeping time, as well as delaying morning departure from their sleeping sites.

In the present study, I observed that rainfall caused the *Nycticebus c. coucang* to leave their sleeping site at later than the usual times of 19:25 - 19:50 hours (pm), and this caused them to arrive at their sleeping sites earlier than usual time of 6:30 - 6:45 hours (am).

Furthermore, studies undertaken by Muller (1995) on Loris species in captivity, demonstrated that tolerance to cold was relatively low, possibly because of its large body surface. Hill (1932) reported that wet fur due to rain or other causes could put individuals at risk of hypothermia when the animal gets its woolly coat thoroughly wet such that it takes considerable time to become dry again.

A novel observation that I made in the field during the present study was when I was able to witness one sub-adult leaving its sleeping tree later than normal due to the rain, and immediately moves towards the electrical wires reaching from one electrical pole to another, in an attempt to dry its fur and get warmth.

7.3.3 Density

The population density of the Slow Loris in this study was computed to be about 13 individuals / km^2 this figure being slightly lower than that reported by Barret (1981) who had studied the Slow Loris in Sungai Tekam Forest Reserve, State of Pahang. Weins (2002), on the other hand, had reported much higher densities, as high as 80 individuals / km^2 in the Segari Melintang Forest Reserve, State of Perak. Nekaris (2007), on the other hand, had reported a median encounter rate of just 0.80 / km^2 for a sample size of n = 15.

There are a number of factors that contribute to the variation in densities reported which are mainly caused by the habitat conditions in which the animal was studied, these varying due to the amount of available resources, to human intrusion into the natural forests, and to human activities such as deforestation, for agricultural, and for housing.

7.3.4 Reproduction

Zuckerman (1932) reported in his work breeding behavior throughout the year, and he captured a total of 146 pregnant females from the wild and found there was a slight increase in pregnancies during the months of September through November. Studies by Izard (1988) revealed that female cycles peaked during the latter half of the year, this being supported in my study that showed males seeking females during this period of the year.

In the present study, I report that the breeding season of the *Nycticebus c. coucang* is long, with annual differences, and may not be as seasonal as had been suggested by Wiens (2002). Slow Loris births were relatively few and the months of parturition occurred around October through January. Nevertheless, high peaks of newly born were observed in the months of February till March, a period as had also been reported by Fitch (2003), and in my study the females (named Timida, and Fala) delivered new offspring in February to March, 2007.

7.3.5 Daily Movement Traveling Crossing-Structures Indicating Spatial Cognition

Several authors have examined the functions of corridors which help to increase movement rates, enhance population densities within the habitat patches, and also to increase gene flow between patches (Beier & Noss, 1998). These were also evident in studies carried out on small mammals (Coffmang, 2001; Fahrig & Merriam, 1985; LaPolla, 1993; Lorenz, 1990).

In this study, evidence is reported of the Slow Loris using crossing structures such electrical wires to move between patches of forest (see Chapter 5). This study reports usage of structural types (high voltage electrical wires, house electrical wires, trees along ponds and bamboos) as movement corridors in

which the majority of the individuals studies (a total of 10 individuals) preferred electrical wire corridors for their inter-biotope movements. The most prominent corridor structure was in fact electrical wire that runs along both sides of the main road and the small side roads within the Cempaka study area.

The evidence provided in this study also showed that these corridor structures between patches of forest facilitated continuity between population groups of *Nycticebus c. coucang* in deforested areas which comprised many different biotopes. Individuals would traverse the structure corridor between patches of forest either by single or return movements in a straight direction. It has been suggested in the literature that corridors provide a source of immigrants to prevent population extinction and inbreeding (Haddad & Baum, 1999; Niemela, 2001; Schmiegelow, 1997). The *Nycticebus c. coucang* in my study area were able to migrate towards isolated, unused or nearby biotope patches, and re-populate by passing on their genes to a new population primarily by using these corridors structures for travelling or ranging movements (see Chapter 5 and Chapter 6).

A few published studies have provided evidence that traveling corridors fulfill their primary function to increase movement between patches (Simberloff & Cox 1987, Rosenberg, 1997). It was suggested by Wilson & Willis (1975) that the possibility of extinction of animals will be lowered when fragments of forest can be connected by corridors. The main assumption is that when there is higher connectivity there will be an increase in the movement rates among patches of forests (Zhang & Usher, 1991). The findings in my study correlate with these previous studies and show an increase in the inter-patch movement rates when the animals used traveling corridors. Ten out of 12 individuals tracked in this study moved more frequently between patches connected by corridors, than patches not connected by corridors. Proportionately, more individuals moved

between connected patches with corridors than between unconnected patches. The propensity to move through corridors was equally the same for both sexes.

The ability of 10 individuals tracked in this study to use traveling corridors within their home range gives rise to questions regarding adaptation, learning processes and spatial cognition of the *Nycticebus c. coucang*. Do these individuals learn by themselves such a detailed network of electrical wire interconnected in different forms in order to bring them to different biotopes? Do they have a spatial cognitive ability or memory of this network and landmarks that were utilized to reach their destination on a daily basis? Or do they have a specific navigation capacity to move from one place to another? How are able to remember which electrical wire to take and in which exact direction to travel?

Research undertaken in past years and focusing on spatial learning has contributed immensely to our better understanding and ability to answer these questions (see, for example, Gallistel, 1989, 1990, 1996; Etiene 1980). Studies carried out by different researchers find a consensus agreement that primates do not move at random in the forest, but in fact they travel towards a specific destination in a straight line (Menzel, 1997, 2002; Garber, 1988, 1989, 2000; Milton, 2000;). Researchers have concluded that animals must be able to represent a spatial memory with landmarks guiding them towards food sources (Janson, 1998, 2000, 2007; Milton, 2000; Biegler, 1996; Etienne, 1996; Rashid & Andrew, 1989).

In my current study, and as shown by the behavior results of the examples I studied, these being, specifically, "Timida", "ECA", "Fala" and adult Cap as described in Chapter 5, my findings strongly suggest that the use of corridors structures are being taught by adults to the young ones, and resulting in spatial

movement patterns continuously being passed on and used by subsequent generations.

Such spatial memory for foraging was reported in prosimian primates by Erhart (2008). Charles-Dominique (1977) in his study concluded that the Lorisine species, *Potto* and A*ngwantibo*, had infants displaying clear learning process acquired from the mother through direct interactions.

Wiens (2002) in his study of the Slow Loris in the State of Perak, on the other hand, and in a clear contradiction to the findings of my study, did not find any evidence of learning processes acquired from the adults through direct observations, or intra-specific interactions in the young individuals.

7.3.6 Home Range and Estimators

My study provides new information on the ranging behavior of *Nycticebus c. coucang* in the wild.

I found MCP % home range sizes for adult male and female, sub-adult and infants were much larger that those previously reported in Peninsular Malaysia by Wiens (2002). The home range sizes reported in my study for adult males ranged from 4.44 to 53.96 ha; for adult females from 11.33 to 41.71 ha; for sub-adults from 6.15 to 20.77 ha; and for infants, in the range 1.56 to 1.79 ha.

The large home range sizes for the *Nycticebus c. coucang* I studied and measured in this study may, I have concluded, be due landscape fragmentation, and which conform to the presence or formation of several biotopes; that is, the study areas were not located in contiguous forest, or unlogged primary forests as was the study area reported in study by Wiens (2002). My study areas, instead,

comprised Semi-Natural Biotopes (Secondary Forest, Belukar/SU, Swamp Fresh Water, fragments of High Canopy Forest, Grass, Ponds, Scrub, Shrubs, and Grass Land) and eight of the Cultural Biotopes (Cleared Areas, New Cleared Areas, Paths in the Cleared Area, Main Road, Small Road, Highway, Agriculture Fields such Rubber Estates, Orchards, and Settlement Areas with their mosque and cemetery). Collins and Barret (1997) mentioned that individuals may range over a wider area to obtain necessary resources in a fragmented habitat. This I believe well explains the sharp differences in the calculated ranging patterns reported in this work when compared with those published in the study by Wiens (2002).

Home ranges illustrate the fundamental measurement of space-use patterns in wildlife ecology (Righton, 2006; Downs et al. 2008). Several studies mentioned the advantages of the statistic KDE (h_{ref}) and (*hlcsv*) which produces accurate estimates with fixes such as 30 to 40; and that it is less sensitive to lack of independence between locations (Kernoham, 2001). Most studies carried out on nocturnal prosimians have, however, used the Minimum Convex Polygon MCP % statistic to understand animal spatial organization (Bearder and Martin, 1980; Radespiel, 2000; Wiens, 2003; Nekaris, 2003). There is, however, one recent study of the *Perodicticus potto* by Pimley (2004) that used both the Kernel (KDE) and MCP % methods, and she concluded that the Kernel method (KDE) gave more reliable size of home range estimates.

My study is the first to use the Kernel estimator method for the *Nycticebus c. coucang,* and I have concluded that this does in fact produce the best estimates for the animal's home ranges. MCP % gives emphasis on external fixes, ignoring any location information recorded within the perimeter of the polygon, and, moreover, the MCP % includes several localities for several individuals. One example highlighted in my study is the adult male ECA where the MCP %

method showed places that this individual actually never used or visited, thus overestimating its home range (and conforming to the findings as carried out by Kenward, 2001). I, therefore, am in agreement that the MCP method performs poorly for home-range analysis, a conclusion which has been reported in numerous other studies (for example, Worton 1987, 1995; Barg, 2005; Franzreb, 2006; Righton & Mills 2006).

My study has showed that the KDE method produces far better estimates of core areas and home ranges, and provides details of its internal structure. However, it ought to be pointed out, that the KDE (h_{ref}) method did overestimate the core areas and home ranges for (4) individuals which I studied. Yet, even taking into account these four cases of over-estimated home range values contours obtained with KDE 95 % (h_{ref}) this estimator performed better when compared with MCP which produced in almost 80% of the cases biologically unrealistic home range estimates.

The best performances in my study were achieved by KDE (*hlcsv*) with contour shapes and home range values closely comparable with the biological reality as observed in the field in my study areas in Bukit Boloh and Cempaka. KDE (*hlcsv*) produced home-range estimates that are more accurate, a conclusion that has also been reported by other field researchers using the same method (such as, Seaman & Powell, 1996; Hemson, 2005).

Lastly, it is also noteworthy to mention, that my study highlights the great performance results produced by the "Animal Movement Analyst Extension" (AMAE) developed for the ArcView GIS (Geographic Information System) software and which I used in analysing my data. It output high precision and realistic analyses and models from my field data. In this finding I concur with similar conclusions expressed by, for example, Selkrik & Bishop (2002).

7.3.7 Spatial Overlap of Individual Home Ranges

Socially, Slow Lorises are in the literature thought to maintain individual home ranges with overlaps primarily on the periphery in males and females (Fitch-Snyder, 1995).

In my study there were large overlaps observed in the home range of adult males with the adult and in sub-adults and infants for group B and C. As for group E, for adult males KRO and ECA, there were 100 % overlaps. In females and their infants or sub-adults the home ranges had 100 % overlap. There was a case in the group C showing a different pattern, the adult female FALA had overlapped home range with one of the sub-adult male GENT by over 65 % (by MCP%) and 65.59 % by the KDE 95 %. These results coincide with those obtained earlier by Wiens (2002).

However, it should be noted that the percentage home range overlapping for intra-groups and inter-groups differed in size and shape between the estimators used, Minimum Convex Polygon (MCP %) and Kernel Density Estimation with reference smoothing factor KDE (h_{ref}) 95 %. The mean percentage overlap between individuals for intra-group and inter-group were higher for MCP % estimator (58.77 % and 15.21 % respectively); whereas when the KDE 95 % estimator was used a lower overlap statistic of 53.63 % for intra-group and 10.60 % for inter group was computed. The difference of values among the two estimators can be explained primarily due to the weak fidelity to biological reality which I observed in the field when reviewing the MCP % estimation method, a critique which I have explained above.

7.4   Molecular Investigation

7.4.1   Limitations of the Microsatellite Marker for Paternity Analysis

For the objective of clarifying the individual relatedness or kinship of the Slow Loris that I studied, I combined radio-telemetry data (Ranging Patterns) with the genotypic profiles. Several samples from other localities were used to measure genetic distances. The investigation of genetic inheritance started with an attempt to study the tandem repeats polymorphism in the animals. Genotype or the DNA sequence from each individual studied was initially done by measuring the variation of microsatellite DNA repeats. The lack of information related to whole genome sequencing for *Nycticebus c. coucang* hindered the process of designing an in-house primer used to amplify the mini or micro-satellite markers for the species. Since no tandem repeat markers have been developed specifically for *Nycticebus c. coucang*, the oligos, or primer pairs, that were initially established for Lemurs and Woolly Monkeys were adopted by me to examine the genotype (Di Fiore, 2004).

Chapter 6, Figure 6.1 shows that all individuals amplified only a single band by polyacrylamide gel electrophoresis across all loci, and this researcher was thus unable to rule out if they are homozygous or heterozygous for each locus. Despite the successful amplification of all three primers used namely, 33228, Mm22, 311, 1118, there no unique allele found for the loci tested.

The possible reason could be due to the narrow locus range which was approximately less than 10 bp differences (for example, for marker 311, the range was 192 – 203 bp). Previous research has found that the narrow repeat length and interruptions of the repeat sequence motif can affect the polymorphism status which might be independent on the successful PCR amplification (Watanabe, 1996). Gastier (1995) reported that microsatellites with

longer repeat sequences were sometimes more polymorphic in more species than those with shorter regions, and should be taken into consideration when adapting primers for cross-species. Another explanation for this could be related to the degree of the resolution of the technique used to segregate the band of each allele if the difference was 1 bp. Since microsatellite DNA loci were repeated sequences of 2 to 5 nucleotides, a detection platform with a 1 bp resolution such as the DNA analyzer might be preferred for a more reproducible results for 1 bp resolution.

7.4.2   Matrilineal Inheritance by D-Loop DNA

My genotypic investigation of the *Nycticebus c. coucang* was expanded to the D-loop mitochondrial DNA diversity, and this further investigation managed to successfully reveal the maternal relationships between the samples being studied.

Unlike the microsatellite tandem repeat marker which was inherited from both paternal and maternal sides and commonly used for paternity testing, this D-loop marker from mitochondrial DNA is, in most organisms, inherited only from maternal lineage. A simple and straightforward technique, D-loop mitochondrial DNA sequence provided a useful tool for the individual's identification (Bowling, 2000). The entire hypervariable segment 1 (mt HVS1) of D-loop was amplified and sequenced for all studied animals (n=26). Based on scholarly search and literature review, this region was shown to have overlapping DNA sequence between the human and primates. The D-loop produced greater polymorphism that enabled correlation to be made for the field data and the genotypic findings based on these markers. Tables 6.3 and 6.6 in Chapter 6 show the data differences in the control region D-loop between the maternal lineages and the reference sample for D-loop region (*Nycticebus c. coucang* Genbank AY875955).

The female inheritance data that were obtained from each individual gave a significant impact to confirm the biological ties that I observed in the field.

The genetic analyses performed in this study was used only for the assignment of matrilineal testing based on haplotype shared within the conserved region of D-loop DNA. The phylogentic tree was calculated using the probability of similarity likelihood based on maximum parsimony. Other researchers have also used this tool to study spacing patterns, dispersal, quantitative evolution and migration in animals. Based on the results obtained, it is here suggested that mitochondrial DNA is more effective than tandem repeats in order to elucidate correlation between filed and genetic relatedness. Tandem repeats analysis by microsatellite amplification (for example, STR) showed undetectable polymorphism and further investigation of cross-species testing by screening more primers developed for other primates could be further improved.

7.4.3 Phylogenetics and Ancestry Inheritance of the Slow Loris from D-Loop Analysis.

The overall strategy of the molecular work was to get information about the amount of gene flow and ancestry inheritance. This would be best described in comparison of the D-loop DNA sequences among representatives of animals obtained from different localities and states.

The *Nycticebus c. coucang* samples showed slightly greater DNA sequence distance and difference among individuals trapped from others states, but still with similarities of ancestrally relationship among them (such as in the samples from Malacca, from Seremban, in the State of Negeri Sembilan, and from Cempaka and Bukit Boloh, both in the State of Pahang). For instance, those individuals in Malacca were shown to be closer, ancestrally speaking, among

themselves than those more spatially distant, such as from Seremban, and from Cempaka and Bukit Boloh (Figure 6.13 and 6.14 in Chapter 6).

7.4.4 Comparison of Kinship analysis from Field and Genetic Data.

For the relationship analysis, the mitochondrial haplotypes of the control region in the *Nycticebus c. coucang* individuals belonging to the same maternal line was provided. The persistence of the same mtDNA haplotype can be explained by the maternal inheritance and by absence of recombination in mtDNA. The tandem telemetry and genotypic kinship analysis for the selected six groups of animals was performed.

Based on field work, it can be successfully determined which of the individuals belonged to the kinship classes, whether unrelated adoption, full siblings or parent-offspring. Further investigation for kinship based on D-loop mitochondrial, undoubtedly confirmed the adoption case (Group A: Infant-Linda), and of parent offspring (group D: Bonita- baby born), such cases as observed in the field serving as a "positive" proof for the biological inheritance as shown by both ecology and molecular techniques. My genetic findings were consistent with the field data for both parent-offspring relationships, and also for parent-offspring exclusion by just using a single mitochondrial marker.

The extent of genetic evidence was more pronounced to answer the assumptions of relationship seen between siblings which was difficult to be ruled out solely by field behaviour observations. This was illustrated in two cases, which were, Group E (IAB, ECA, KRO) and Group A (male Boloh, Yuri). Whilst in Group E, ECA behaviour towards IAB and KRO either as a parent or as an adult sibling were almost indistinguishable based on just field observations. Although all three of them have overlapping home ranges, ECA actually had a home range twice as large as the other two animals, and ECA was also found to be more tolerant

towards these others. It was only by using genetics that it was possible to rule out that these 3 animals were siblings with parental ties of ECA towards IAB and KRO. In Group A, Male Boloh and Yuri relationship was confirmed by D-loop analysis as siblings, a relationship that by ecology alone was not sufficient to strictly rule out an explanation whether they were siblings or just individuals previously living within overlapping home ranges.

7.4.5 The Adoption Case Observed In the Wild

Crandall (1964) reported a case of parental care for a Slow Loris (*Nycticebus coucang)* in captivity. The fact that the circumstances in which infant adoption occurs in the wild can be successfully reproduced in the laboratory suggested that primate females have the potential to adopt unrelated infants (Maestripieri, 2001). Interest in infants, especially very young ones, by group members other than the mother seems to be widespread among nonhuman primates.

In this study, a special case was witnessed in which an infant was adopted in the wild by a female adult *Nycticebus c. coucang*, Such a finding has not been previously reported in the literature in the genus of pro-primates living in free-ranging conditions, and this was proven based on both radio-telemetry studies carried out in the field and through D-loop mitochondrial DNA sequences (see Chapters 5 and 6 respectively).

Adoption has been described as a vehicle for the transfer of parental behaviour patterns across lineages (Avital & Jablonka 1994) and for the "phenotypic cloning" for some of the parents behaviors that could reflect the transfer of food preferences, foraging techniques, mate preferences, predator avoidance techniques, and niche preference (Harris & Murie 1984; McFarland 1987; Cheney & Seyfarth 1990; Terkel, 1996). Indeed, the adoptee individual in this

study managed to learn and maintain the acquired behaviors especially in reference to the choice of sleeping sites and food preferences.

Alloparental behavior and adoptive behavioral patterns seem diverse. Three general patterns are considered: nursing, carrying, cuddling, and protecting (Thierry & Anderson, 1986). Riedman (1982) proposed that mammals and birds were most likely to demonstrate alloparenting or adoptive behaviors. Care for alien young, particularly by female mammals was frequently observed in groups whose members were related by matrilineal descent (Riedman, 1982).

My study has proven that not all of the animals studied were related through matrilineal descent as the adoptive adult female displayed natural behaviours such as nursing, carrying, cuddling and protection behavior patterns in captivity as well as in the field.

An alloparent would be an individual that provided partial care of conspecific young not its own offspring. This role may change to an adoption role if biological parents die (Wilson, 1974). The fact that the adoptive female brought the adoptee infant to a safe location by crossing through several biotope hundreds meters away from the release point, and did not abandon the infant in a open location, this being clear proof of a parental role taken by this non-conspecific adult female.

Adoption of orphaned nonhuman primates had been documented in anthropoid species in captivity (Thierry & Anderson, 1986; Hardy, 1976). It has been argued that the observations in the wild of infant adoption were too few for this phenomenon to have any biological significance (Maestripieri, 2001). Nevertheless, It must be emphasized, that infant adoption is probably under-

reported (Maestripeiri, 2001), and this is especially in this case of the *Nycticebus c. coucang* because so few field studies that have been done on them.

My finding on infant adoption is, therefore, a valuable addition to the field knowledge of the free-ranging Slow Loris (*Nycticebus c. coucang*).

7.4.6 Radio-Telemetry, Genetics, and GIS Technologies

Authors such as Di Fiore (2003) explain that studies in the field of behavior and ecology of primates have contributed to the understanding of the biology of these species, but long-term studies in the wild cannot fully elucidate social systems such as dispersal patterns, relatedness, and the effective genetic mating system. Moreover, observational studies alone cannot fully evaluate the effect of kinship on shaping patterns of social behavior and examination between individual behavior and reproductive success (Di Fiore, 2003).

In the present field study I have been able to correlate those statements raised by directly incorporating molecular examination.

Observations based merely on the data obtained in the field and radio-telemetry studies would have given uncertain answers of relatedness with some unequivocal interpretation of some field events. Hence, I concluded that the simultaneous realization of these research objectives necessarily required the combination of both radio-telemetry tracking combined with Biotope Mapping (using GIS technology) for ecological context analysis, as well as molecular laboratory work.

Over the last decade conservation biologists have been using these newly developed molecular techniques to address fundamental and biological questions in field ecology (Haig, 1998; Parker, 1998). Recent literature shows

that studies combining ecology and genetic information are now emerging (Hughes, 1998). Nevertheless, researchers often do not consider using molecular data despite the potential usefulness of these techniques for studies related to the ecology.

Moreover, a perception that there is a division between these two disciplines (ecology and genetics) still persists (see, for example, Deyoung, 2005).

I can now conclude that those objectives of research as identified in my present study could have not been answered within only one discipline. I have shown in this report that I had to innovate by integrating a number of diverse approaches from several disciplines. These included the components of Molecular Techniques, Remote Sensing and Field Biotope Mapping, and GIS Mapping, all of which in combination substantially improved on the interpretive value of the traditional Radio-Telemetry methods as used in animal studies.

7.5 Conclusions

The development of biotope maps for the study areas using a GIS program allowed for the superimposition of a vast amount of data collected in the field, and which enhanced visual analysis.

The total surface area of biotopes in my study areas were in Bukit Boloh 201.535 ha, and in Cempaka 642.942 ha. Semi-natural biotopes in both study areas totaled 230.9184 ha (28 %) while that of cultural biotopes covered 613.5594 ha (72 %).

With respect to the number of sites, there were 116 semi-natural, and 276 mixed biotope sites. The size of each biotope site varies. The largest semi-natural

biotope is the Belukar forest at Bukit Boloh and Cempaka with a surface area of 42.33 ha and 86.113 ha respectively. The largest biotope in the cultural group is Rubber Plantation in Bukit Boloh and Cempaka, with 58.06 ha and 266.88 ha respectively.

It was revealed in this study that the Slow Loris movement rates were affected by traveling corridors due to consistent use of different biotopes within the home range. Inter-biotope movements were detected for all radio tagged individuals.

There were no observed differences based on gender or age differences, nor elevation and movement patterns among individuals. Elevation ranges were from 68 to 118 meters in altitude in Bukit Boloh, and from 56 to 89 meters in altitude in the Cempaka area. Slow Loris movement was mostly at 10 - 15 m heights (43.2 %), resting at 15 - 20 m (43 %), and sleeping at 5 - 10 m (49 %).

Activities commenced at 18:55 – 19:25 hours (pm) and ended 06:57 – 07:27 hours (am), with peak hours between 18:55 – 21:00 hours (pm), 23:00 – 01:00 hours (pm-am), and 03:00 – 06:00 hours (am).

The mean daily distance traveled by adult males during a single a night was recorded as between 280.05 - 1077.06 m, for adult females between 311.15 - 978.63 m, for sub-adults between 76.17 - 568.11 m, and for the two infants between 50.5 - 158 m.

Maximum distance traveled in a straight line on a single night was 1562.48 meters. 676 was the total number of sleeping sites visited during the study period. Individuals slept alone, or in duo, but three animals were never recorded sleeping on the same tree.

For movement corridors the Slow Loris used structural types such Electrical Wires, House Electrical Wire, Trees growing along ponds and Bamboos. Furthermore, traveling corridors used were similar among both sex classes, there being no observable differences.

There were differences generated when the two methods MCP % and 95 % KDE were used to model home ranges.

Using MCP 100%, the home range of adult males varied from 4.44 - 53.96 ha, adult females from 11.33 - 41.17 ha, sub-adults from 6.15 - 20.77 ha, infants from 1.56 - 1.79 ha.

Using the KDE href (Kernel 95 %) analysis the home range results for adults males were from 4.40 - 51.32 ha, adult females from 7.54 - 45.16 ha, sub-adults from 4.57 - 18.02 ha.

Male individuals had larger home ranges than females with 34.61 ha for adult males, 21.48 ha for adult females, 13.46 for sub-adults and 1.68 for infants using MCP %. The mean percentage overlap between individuals for intra-group and inter-group were higher for the MCP% estimator (58.77% and 15.21% respectively); whereas the KDE 95% estimator overlap was lower with 53.63% for intra-group and 10.60% for inter-group.

I concluded that the best performance was achieved by the KDE LCSV estimator which had contour shapes and home range values closely comparable with direct observations made in the field.

A novel approach used in this study was to combine the results of field radio-telemetry with laboratory molecular analysis.

The social structure model of the species obtained in the field as well as the background from where the individual samples were obtained is consistent with the presence of close kinship among groups.

In total, 13 distinct mitochondrial DNA sequences were found to have similar nucleotides with 2 or 3 individuals from the group or outside the group. In those groups, the adult males, or females, infants, and subadults living in the group or in a nearby area shared the same DNA sequence. This is in agreement with the general rule that mtDNA is inherited maternally. Although, the sample size in this study is admittedly small, the results support the assumption inferred from the study field of these groups, as well as from the samples were taken in the Zoo Malacca, and the Perhilitan (Wildlife Department) Center.

This project has contributed insights into important behavioral and molecular processes operating within the Malaysian *Nycticebus c. coucang* populations. I expect my findings to encourage other researchers to study more extensively and deeply questions about the Slow Loris (*Nycticebus c. coucang*) ranging patterns that, I argue in this research, are now being shown not to be "typical throughout the species range" in South-Asia. More studies, and especially longer term studies, are, therefore, required.

My findings also have potentially important implications for the conservation of the *Nycticebus c. coucang* in Peninsular Malaysia, and I expect my research to have contributed to improve considerably on the current general lack of the biological understanding of this understudied species, and, therefore, to assist in furthering the cause of conserving this elusive, rare, and distinctive wild Malaysian primate.