STUDIES OF BEHAVIOUR AND MOLECULAR CHARACTERIZATION OF NORTHERN PIGTAILED MACAQUE (*Macaca leonina* BLYTH, 1863) IN MIZORAM, INDIA

BY

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DECLARATION

I, Phoebe Lalremruati, hereby declare that the subject matter of this thesis entitled "**Studies of behaviour and molecular characterization of Northern Pigtailed Macaque (*Macaca leonina* Blyth, 1863) in Mizoram, India**" is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other university/Institute.

This is being submitted to the Mizoram University for the degree of Doctor of Philosophy in Zoology.

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CERTIFICATE OF SUPERVISOR

I certify that the thesis entitled “Studies of behaviour and molecular characterization of Northern Pigtailed Macaque (*Macaca leonina* Blyth, 1863) in Mizoram, India” submitted to Mizoram University for the award of the degree of Doctor of Philosophy in Zoology by Phoebe Lalremruati is a record of research work carried out during the period of 2015 to 2019 under my guidance and supervision, and that this work has not formed the basis for the award of any degree, diploma, associateship, fellowship or other titles in this university or any other university or institution of higher learning.

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ERTIFICATE OF HOD

This is to certify that Ms. Phoebe Lalremruati, a Ph.D Scholar, Registration No. MZU/Ph.D/927 of 22.04.2016 has worked on the thesis entitled "Studies of behaviour and molecular characterization of Northern Pigtailed Macaque (*Macaca leonina* Blyth, 1863) in Mizoram, India." She has fulfilled all the criteria prescribed by the UGC (Minimum Standard and Procedure governing Ph.D Regulation). She has fulfilled the mandatory publication (publication enclosed). It is also certified that the scholar has been admitted in the department through an entrance test followed by an interview as per Clause 9 (i) and (ii) of the UGC Regulation, 2009.

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CHAPTER I

GENERAL INTRODUCTION

Primate is the third most diverse order of mammals after rodents (Rodentia) and bats (Chiroptera) (Napier and Napier, 1967). On an evolutionary time scale, primates are of very recent origin. The first known primates appeared around 70-65 million years ago. The first primates were probably small arboreal, quadrupedal omnivores weighing around 150 grams and obtaining their food on the ground and in the lower levels of tropical forests (Fleagle, 1988; Groves, 1993). Members of the order Primate show a remarkable range of size and adaptive diversity. The smallest Primate is Madame Berthe's mouse lemur (*Microcebus berthae*) of Madagascar which weighs some 35 grams; the most massive certainly being Eastern gorilla, weighing over 200 kg (Napier and Napier, 1967). The living primates can be divided into Prosimians and Simians or Anthropoids. Prosimians comprises of lemurs, lorises, bushbabies and tarsiers. Simians or Anthropoids comprises of monkeys, apes and men. The major distinctions between prosimians and the anthropoid are in their sensory anatomy and physiology. Moreover, at the center of these distinctions, the majority of the prosimians are nocturnal and anthropoids are diurnal. Prosimians possess relatively small brain, relatively weak neuromuscular control over their hands and digits as compared to the anthropoids (Bishop, 1964). They have relatively large eyes, sensitive nocturnal vision, elaborate tactile hairs and a well-developed sense of smell (Bearder, 1987). The anthropoid primates are advanced phylogenetically and sense organs and perceptual abilities are adapted accordingly.

Monkeys are commonly referred to as Old World monkeys (OWM), whose extant representatives live in Africa and Asia, and New World monkeys (NWM) are distributed in Central and South America. New World monkeys are represented by two families, namely Callitrichidae, that includes the tiny marmosets and tamarins and Cebidae, a diverse group which contains species such as howler monkeys (*Alouatta* spp.), titi monkeys (*Callicebus* spp.), squirrel monkeys (*Saimiri* spp.), capuchins (*Cebus* spp.), spider monkeys (*Ateles* spp.) and woolly monkeys (*Lagothrix* spp.)

Old World monkey represents a larger group and included monkeys, langurs and apes. Old World Monkey comprises of two subfamilies, the Cercopithecinae and Colobinae. Cercopithecinae includes monkeys with simple digestive systems and cheek pouches, and Colobinae includes monkeys with guts anatomically specialized for feeding on leaves. The members of the subfamily Cercopithecinae eat a variety of foods and have cheek pouches. This subfamily comprises of three genera, namely guenons (*Cercopithecus* spp), macaques (*Macaca* spp.) and baboons (*Papio* spp.). Members of the subfamily Colobinae are loosely referred to as 'leaf-eating monkey' and have large complex stomachs containing cellulose-digesting bacteria. The subfamily Colobinae comprises of 11 genera and about 41 species (Thorington and Groves 1970; Oates and Trocco, 1983; Brandon-Jones, 1984; Napier and Napier, 1985; Weitzel and Groves, 1985).

Primates mostly live in groups of different sizes and compositions. The groups are more stable and the most striking characteristics of the primates are their sociality. Despite the debating issues on evolution of group living primates, it is clear that group living inevitably increases competition for limited food resources (Wrangham, 1980; van Schaik, 1983). Diurnal primate lives in the group while the nocturnal species are largely solitary (van Schaik and van Hoof, 1983). The primates such as baboons and macaques live in the largest group that comprises as much as 100 individuals (Dunbar, 1988). Many species among the old-world monkeys are usually found in one-male unit groups, consisting of a single breeding male and number of adult females (Mohnot and Srivastava, 1992). The rhesus macaques, stumptailed macaques, assamese macaques and pigtailed macaques of northeast India and savanna baboons of Africa usually live in multi-male multi-female groups. The group consists of several breeding males and a larger number of adult females. It was once thought that the presence of several males in a troop served to protect females and juveniles from attack by predators. This hypothesis has little evidence in support (Eisenberg et al., 1972). Another explanation for the occurrence of multi-male troops is that they occur where female groups are too large or too widely dispersed to be feasible for a dominant male to monopolize and have exclusive control over female’s reproduction. Additionally, he is not able to keep the rival males away from receptive females. Many adult males in the group may enhance the defense of the group’s home range, and its competitive advantage in inter-group encounters (Srivastava and Dunbar, 1996).

Environment, reproduction and parental care are the important aspects in evolution of primates. These are associated with increased and prolonged maternal care, delayed sexual maturity and extended life-span, and a progressive dependence on fruit and/or foliage, with a reduction in the proportion of animal matter in diet (Jolly, 1985; Napier and Napier, 1985). During evolution, the early primate diverged progressively from their insectivorous ancestors, they required fewer teeth but a bigger tooth surface area for grinding the fruit and foliage that became a larger part of their diets (Fleagle, 1988). Primates are confined in their distribution to the tropical and sub-tropical regions (23^0N and 23^0S) of Africa, Asia and Madagascar and Central and South America (Gupta, 2000). It is very likely that their dependence on diets consisting largely of fruits, shoots or insects that are scarce during winter in temperate regions restricts primate distribution to the tropics and sub-tropics (Fossey, 1983). Body size alone is a good indicator of primate diet since all insectivorous primates are small in size (Martin, 1984). Folivorous species tend to be larger than their frugivorous relatives, hence the folivorous siamang is larger than the frugivorous gibbon and the folivorous gorilla is bigger than the frugivorous chimpanzee. Folivorous primates are usually more sedentary than frugivorous ones. They also have smaller home ranges and their population densities are higher (Ross, 1992).

Primates have advanced cognitive abilities. Some make tools and use them to acquire food and for social displays (Boesch and Boesch, 1990; Westergaard and Lundquist, 1998), some have sophisticated hunting strategies requiring cooperation, influence and rank (de Waal and Davis, 2003), they are status conscious, manipulative and capable of deception (Parr et al., 2000), they can recognize kin and conspecifics (Parr and de Waal, 1999) and they can learn to use symbols and understand aspects of human language including some relational syntax and concepts of number and numerical sequence (Itakura and Tanaka, 1998; Call, 2001; Gouteux et al., 2001). Research in primate cognition explores problem solving, memory, social interaction, a theory of mind, and numerical, spatial, and abstract concepts (Tomasello and Call, 1997). Comparative studies show a trend towards higher intelligence going from prosimians to New World monkeys to Old World monkeys, and significantly higher average cognitive abilities in the great apes (Deaner et al., 2006; Reader et al., 2011). Lemurs, lorises, tarsiers, and New World monkeys rely on olfactory signals for many aspects of social and reproductive behaviour. Specialized glands are used to mark territories with pheromones, which are detected by the vomeronasal organ; this process forms a large part of the communication behaviour of these primates. In Old World monkeys and apes this ability is mostly vestigial, having regressed as trichromatic eyes evolved to become the main sensory organ (Liman and Innan, 2003). Primates also use vocalizations, gestures, and facial expressions to convey psychological state (Egnor et al., 2004). Facial musculature is complex in primates, particularly in monkeys and apes. Like humans, chimpanzees can distinguish the faces of familiar and unfamiliar individuals (Burrows, 2008). Hand and arm gestures are also important forms of communication for great apes and a single gesture can have multiple functions (Pollick and de Waal, 2007).

Hunting, emergent diseases, habitat conversion and fragmentation are the main reasons for the global decline of primate populations (Oates, 1996; Cowlishaw and Dunbar, 2000; Nunn and Altizer, 2006), and as a consequence of these activities, more than half of the world’s primate species are currently threatened and some have gone extinct (Chapman and Peres, 2001). Several characteristics of primates may influence their ability to live in forest fragments. Home range size is frequently cited as an influencing factor (Lovejoy et al., 1986). The impact of habitat alteration on nonhuman primate populations depends on the nature and scale of the disturbance, and the extent of the primates' ecological and behavioural flexibility in response to changes, which can vary among taxa (Marsh and Wilson, 1981; Johns and Skorupa, 1987; Marsh and Mittermeier, 1987; Singh and Vinathe, 1990; Pearl, 1992).

India represents parts of four biodiversity Hotspots, which are Western Ghat, Eastern Himalaya, Indo-Burma region and Sundaland. Primates form an integral part of the biodiversity India and cognizable link between human and nature. India hosts 26 species of primates (Solanki, 2015). Northeast India comprising the states of Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, and Tripura falls in the Eastern Himalayan ecoregion, a priority biodiversity hotspot (Champion and Seth, 1968). At the confluence of the Indo-Malayan and Palearctic biogeographic realms, the region contains a profusion of habitats characterized by diverse biota with a high level of endemism. The region is also the abode of approximately 225 of India's 450 tribes, the culture and customs of which have an important role in understanding biodiversity conservation and management issues. Northeast India has the highest primate diversity in India. The 46.15% (12 species) of the total Indian primates are found in this northeastern region (Molur et al., 1998; Srivastava, 1999; Srivastava and Mohnot, 2001; Biswas et al., 2011; Solanki, 2015) and are represented by capped langur (*Trachypithecus pileatus),* golden langur (*T. geei),* phayre's leaf monkey (*T. phayrei),* slow loris (*Nycticebus bengalensis),* rhesus macaque (*Macaca mulatta*), assamese macaque (*M. assamensis),* northern pigtailed macaque (*M. leonina),* stumptailed macaque (*M. arctoides),* Arunachal macaque (*Macaca munzala*), white cheeked macaque (*Macaca leucogenys*), western hoolock gibbon (*Hoolock hoolock)* and eastern hoolock gibbon (*Hoolock leuconedys*). Of these 12 primate species in northeastern region, 8 are found in Mizoram, such as *Macaca mulatta, Macaca assamensis, Macaca leonina, Macaca arctoides, Trachypithecus pileatus, Trachypithecus phayrei, Nycticebus bengalensis and Hoolock hoolock* (Sawmliana, 2009).

In order to prevent the extinction of a significant percentage of primates, empirical information about these species and their habitats was required (Srivastava, 2006). Habitat loss is the principal threat to wild primate populations in Northeast India. Habitat loss results from clear cutting for settlements and agriculture, and forests are also selectively logged for fuelwood and construction material and exploited for natural products. The population pressure on natural habitats, combined with hunting, and live capture has driven numerous species to the brink of extinction. The hunting of primates in Northeast India takes place for a number of reasons, but by far the most important is for food. Although hunting is prohibited by the Wildlife (Protection) Act of India of 1972 (amended 2002), its enforcement is usually nonexistent in the remote areas. In areas where the hunting of primates for food is common, it can represent a threat even more severe than forest destruction. In Arunachal Pradesh, Mizoram and Nagaland, for example, there are large tracts of primary forest remaining where primate populations have been either exterminated or pushed to the brink of local extinction by excessive hunting. Primates may also be killed when they raid and damage crops (Srivastava, 2006).

The northern pigtailed macaque (*Macaca leonina* Blyth, 1863) is found in Bangladesh, Cambodia, China, India, Laos, Myanmar, Thailand, and Vietnam. Traditionally, the northern pigtailed macaque was considered a subspecies of the southern pigtailed macaque, however they are now classified as individual species

(Choudhury, 2003). In India, it is found in the south of the Brahmaputra River, in the northeastern part of the country. Its range in India extends from Assam and Meghalaya to eastern Arunachal Pradesh, Nagaland, Manipur, Mizoram and Tripura (Choudhury, 2008). It is a threatened species and belongs to the Vulnerable category as per IUCN Red List Category (2004), Schedule II as per CITES Appendix, and Schedule II (Part I) as per Indian Wildlife (Protection) Act, 1972. (Sharma et al., 2015). Pigtailed macaques are found in tropical semi-evergreen forest, tropical wet evergreen forest, tropical moist deciduous forest and occasionally are found in swamp and secondary forests (Crockett and Wilson, 1980). They are found at an elevation of 50 to 1700m. They prefer undisturbed forests and are found in the highest densities in intact rainforests. (Choudhury, 1989; Brandon-Jones, 2002; Chetry et al., 2002; Choudhury, 2003). Pigtailed macaques are predominantly arboreal, diurnal and highly frugivorous, with 74% of their diet consisting of fruit, but they also consume a wide variety of foods including insects, seeds, young leaves, leaf stems and fungus (Crockett and Wilson 1980; Caldecott 1986). The troop size ranges from 11 to 33, with a mean size of approximately 21 individuals (Choudhury, 2008, Sharma et al., 2008). Pigtailed macaques are particularly adapted at raiding agricultural fields and obtaining coconuts from oil palm plantations, papaya, corn, and cassava. They are stealthy crop raiders, sneaking silently into a garden one at a time, with one acting as a lookout and calling an alarm vocalization if humans are seen. Pigtailed macaques are especially likely to raid crops during rainstorms, when farmers are inside, away from their crops (Crockett and Wilson, 1980). In some areas of the Malay Peninsula, farmers keep and train pigtailed macaques to retrieve coconuts and fruits from cultivated trees (Crockett and Wilson, 1980). The northern pigtailed macaques decline in population in India due to several factors such as habitat loss and forest fragmentation, predators, local trade for bones, meat for food and medicine (Choudhury, 1989; Brandon-Jones et al., 2002; Chetry et al., 2002; Choudhury, 2003). Conservation Assessment and Management Plan (C.A.M.P.) Workshop Report, (2003) reported that northern pigtailed macaque should be upgraded from Schedule II to I (WPA, 1972) to ensure more legal protection. Brandon-Jones et al. (2002), Chetry et al. (2002), Choudhury (1989) and Choudhury (2003) reported that detailed survey with proper documentation is urgently needed in northeastern India.

Understanding the behaviour of animals is essential for the development of strategies that are required for the management of a species. Behavioural biology includes evolutionary cause of behaviour, mechanism (relation of favoured genetically transmitted pattern), the selective advantages of behaviour (adaptive significance), and ontogenic development of behavioural traits (Tinbergin, 1951). The study of non-human primates has a close bearing on the understanding of human, social and psychological problems which are widely realized. In addition to its relevance to the study of social evolution, the study of primates is important in a number of other fields such as medicine and agriculture (protecting crop from primate pests). The study of non-human primates has contributed to the understanding of basic biological phenomena, human disease, social behaviour and life styles of human societies. Information on various aspect studied on captive animals could imply to wild populations for monitoring them. Understanding the differences in adaptability between the species living in natural habitats versus those that are living in captive conditions can lead to an understanding of how animals improve adaptability as the environment change. In spite of the rich heritage of non-human primates' populations in India, most of these species are in their way to extinction. Therefore, captive condition could serve as the Centre for Conservation of a species genome. This study was undertaken for better understanding of northern pigtailed macaque's behaviour in different aspects, and to obtain information which may help to improve management of species and to develop conservation measures both for in-situ and ex-situ conditions.

CHAPTER II

REVIEW OF LITERATURE

Several studies and researches have been done on different mammalian species including non-primates regarding the correlation between the food preferences and their nutritional contents (Laska et al., 2000a, 2000b). The food selection by primates correlated negatively with the content of plant secondary compounds such as phenolics, alkaloids or tannins that inhibit digestion of proteins or polysaccharides or are toxic (Glander, 1982; Waterman, 1984; Wrangham et al., 1998). Laska et al. (2000a, 2000b) observed a positive correlation between the food preferences displayed by captive spider monkeys (*Ateles geoffroyi*) and the content of total energy in the foods used. Additional studies that employed the same approach showed that, like the spider monkeys, captive squirrel monkeys (*Saimiri sciureus*: Laska, 2001), and pacas (*Agouti paca*: Laska et. al., 2003) displayed food preferences that correlate highly significantly with total energy content. Conversely, pigtailed macaques (Laska, 2001) prefer foods with high contents of total carbohydrates but not foods that are high in total energy content or in other sources of metabolic energy such as proteins or lipids. Captive white-handed gibbons (*Hylobates lar*) showed preferences for food which are high in fructose, glucose and total carbohydrates content of the food items (Jildmalm et. al., 2008). Pigtailed macaques and white handed gibbons include a high proportion of carbohydrate rich foods in their natural diet (Chivers, 1984) and show clear preferences towards ripe fruits, which typically have the highest content of soluble carbohydrates (Bollard, 1970; Simmen et. al., 1999). Soluble carbohydrates have been reported to influence food choice by a wide variety of frugivorous vertebrates, including birds, bats and primates (Johnson et. al., 1985; Janson et. al., 1986; Herrera, 1987; Wrangham et.

al., 1991; Leighton, 1993; Wendeln et. al., 2000). Visalberghi et. al. (2003) observed in socially housed tufted capuchins (*Cebus apella*) that their food preferences were positively correlated with the glucose and fructose content of the foods, and negatively with their total and insoluble fiber and phosphorus contents.

It is reported that primates can communicate information about their social and physical environment in addition to emotional states or arousal (Gouzoules et al., 1995). Description of the gestures displayed by several species of nonhuman primates has been done by several researchers (Darwin, 1872; Altmann, 1962; Hinde and Rowell, 1962; van Hooff, 1962, 1967; Andrew, 1963; Kaufman and Rosenblum, 1966; van Lawick-Goodall, 1972; Redican, 1975; Marriott and Salzen, 1978; Weigel, 1979; Kirkevold et al., 1982; Preuschoft, 1992; Tomasello et al., 1994). Pigtailed macaque has been reported to display rich gestural communicative repertoire which has long been recognized (Cole, 1963; Bobbitt et al., 1964; Kaufman and Rosenblum, 1966; Goosen and Kortmulder, 1979). Goosen and Kortmulder (1979) recorded 19 different facial expressions in pigtailed macaque, the highest number of facial expressions ever observed on any monkey species. One of the most common gestural signals in pigtailed macaque has been reported to be pucker or pucker face. Pucker has been known by different names (Bernstein, 1967), known as flehmen face (van Hooff, 1962), len (Bobbitt et al., 1964), protruded lips face (van Hooff, 1967), and jaw thrust (Kaufman and Rosenblum, 1966), which is frequently displayed in this species. Pucker has only been rarely observed in other primates (in rhesus macaques: van Hooff, 1967; in liontail macaques, *Macaca silenus*: van Hooff, 1967; in longtail macaques, *Macaca* *fascicularis*: Shirek-Ellefson, 1972). Lipsmack and bared-teeth were also among the most frequent gestural signal in pigtailed macaques (Maestripieri, 1996). Maestripieri (2005) observed 12 types of gestural signals in stumptailed macaque (*Macaca arctoides*), 8 types of gestural signals in pigtailed macaque (*Macaca leonina*) and only 4 types of gestural signals in rhesus macaques (*Macaca mulatta*). Pucker, lipsmack, bared-teeth, present and mount were reported to occur in high frequency in macaques (Maestripieri, 1996, 2005). Lalremruati et al. (2017) recorded 9 types of gestural signals in assamese macaque and 8 types of gestural signals in rhesus macaque. 24 gestural signals were exhibited by bonnet macaques in the wild (Gupta, 2015).

Grooming is a common and well-documented behaviour in primates (Sparks, 1967; Goosen, 1987). Researchers have reported that some species of nonhuman primates invest at least one-fifth of their time in grooming (Dunbar, 1991; Shutt et al., 2007). However, grooming has been reported to be an infrequent behaviour in spider monkeys (*Ateles geoffroyi*). On an average, individuals of spider monkeys allocated only 2.5% of their daily activity to grooming (Ahumada, 1992). Freeland (1981), Sánchez- Villagra et al. (1998), Tanaka and Takefushi (1993) and Zamma (2002) suggested that grooming plays an important role in ectoparasite removal and maintaining hygiene. Boccia et al. (1989) and Aureli et al. (1999) also reported a reduction in heart rate in individuals receiving grooming. Long-tail macaques (*Macaca fascicularis*) were found to display less self-directed behaviour soon after they were groomed (Schino et al., 1988). Shutt et al. (2007) have recently shown that in Barbary macaques (*Macaca* *sylvanus*), the giving rather than the receiving of grooming is associated with lower stress levels in the longer term, as measured by faecal glucocorticoid concentrations. Schino and Alessandrini (2015) reported in Japanese macaques (*Macaca fuscata*) that grooming is associated with decreased scratching rates, reduction in the displacement activities (a behavioural indicator of anxiety), and decreases the heart rate. Kumar and Solanki (2014) reported in capped langur that grooming maintains group integrity in which intra-group alliances might be crucial for individual reproductive success. Social grooming manipulates over reproduction of individuals and also acts as an essential tool for formulating survival strategies of the species. Reichard and Sommer (1994) observed the distribution of grooming bouts and reported that allogrooming occured more in the upper body areas than lower body parts. The concentration of allogrooming on upper body regions- such as head, shoulders and arms, probably reflects a hygienic funtion. Seyfarth (1977) observed that high-ranking animals receive more grooming than others, and the majority of grooming occurs between females of adjacent rank.

Parental care is critical for infant survival in mammals (Nicolson, 1991). Mothers play an important role in attaining independence by their offsprings. Infants of white-headed langur cling to their mother on the first week, and then as early as five months of age, mothers start regularly rejecting an infant from nursing or being carried around. At the age of 21 months, they are frequently rejected and weaning is allowed by the mother. They start exploratory play around two months, which then turns into social play after three months of age, which is continued to around 16 months of age (Zhao et al., 2008). Maestripieri (1994) observed that rhesus macaque mothers were more encouraging for the infant independence and less protective than pigtailed macaque mothers. Similarly, Lalchhuanawma (2013) observed that rhesus macaque mothers broke contact with the infant more often than assamese macaque mothers, which suggest that rhesus mothers were more encouraging of their infant independence and less protective than assamese macaque mothers. Captive red-shanked doucs could locomote independently by 8-13 weeks of age (Yeong et al., 2010). White-headed langurs start leaping around 6-7 weeks of age (Zhao et al., 2008). Juveniles have the skills, strength, and coordination to locomote like adults, although they have a larger locomotor repertoire than adults (Dunbar and Badam, 1998; Covert et al., 2004; Workman and Covert, 2005).

Stuart et al. (1990) reported 48% infection with gastrointestinal parasites in red howler monkeys. The observed gastrointestinal parasites were *Strongyle* sp., *Trypanoxyuris minutes*, *Controrchis biliophilus* and *Isospora* sp. oocysts. Modi et al. (1995) observed the occurrence of *Ascaris* sp., *Strongyloides* sp., *Ancylostoma* sp., *Fasciola* sp., *Oesophagostomum* sp., *Paramphistomum* sp. and *Trichuris* sp. in gibbon. Kimberley et al. (2004) reported infection with *trichuris trichiura Ancyclostoma* sp., *ascaris* sp., *Prosthenorchis elegans, Strongyloides stercoralis* and *Schistosoma mansoni*. for the different monkeys i.e. red howler monkeys (*Alouatta seniculus*), brown titi monkeys (*Callicebus brunneus*), brown capuchins (*Cebus paella*), night monkeys (*Aotus vociferans*), whitefronted capuchins (*Cebus albifrons*), spider monkeys (*Ateles bezlebuth chamek*), saddleback tamarins (*Saguinus fuscicollis*) and squirrel monkeys (*Saimiri sciureus*). Lim et al. (2008) observed 54.4% rate of infection in primates in captivity, which includes, *Cryptosporidium* sp., hookworm, *Balantidium coli,* ascarid and *Trichuris* sp. Cordon et al. (2008) studied the gastrointestinal parasites in Zoo animals and the seasonality of the parasitism, 72.5% of animals were infected with one or more parasites. The most common endoparasites found include *Eimeria* sp, *Strongyloides* sp., *Cryptosporidium* sp., *Trichuris* sp., *Cyclospora* sp. and *Isospora* sp. Multiple parasitic infestations were common and 70% animals were infected with at least two parasites. Opara et al. (2010) and Thawait et al. (2014) observed the overall prevalence of parasites to be 76.6% and 60% respectively, in captive animals. Li et al. (2015) and Nath et al. (2012) reported low incidence of infection with gastrointestinal parasites (26.51% and 13.63% respectively) in the captive primates. *Trichuris* sp. and *Entamoeba* sp. were the most commonly found parasites. Researchers have also reported higher parasite infection during the dry season (van Geldorp and van Veen 1976; Appleton and Henzi 1993; Bakuza and Nkwengulila, 2009). Margono et al. (2013) reported only helminth infection in slow loris, but not protozoan. Nematodes have been detected in higher prevalence in primates including *Trichuris*, *Strongyloides*, *Strongylus*, *Gongylonema*, *Oxyuris*, *Enterobius*, *Physaloptera*, *Filaria*, *Spirura*, *Microfilaria*, *Breinlia*, *Pterygodermatides* (Sutherland-Smith and Stalis, 2001).

Mate selection is a very conspicuous and complex phenomenon in all the animals including primates. There are several patterns of mate choices in primates, such as male coloration (Waitt et al., 2003), genital swelling (Fitzpatrick et al., 2015; Fitzpatrick and Servedio, 2016) and male mate selection due to female fecundity (Jones et al., 2001). Sexual selection theory predicts that female primates are the more selective sex because of their parental investment (Trivers, 1972).

Males are less selective in their sexual preferences than females (Parga, 2006). Cowlishaw and Dunbar (1991), Mainguy et al. (2008), Rodriguez-Llanes et al. (2009) and Packer et al. (1991) reported that dominance hierarchies of male primates often play a major role in male mating opportunities, and also reported a positive correlation between male dominance rank and mating activity. Alberts et al. (2006) and Boesch et al. (2006) observed that males of higher dominance rank sire more offspring than subordinates among the primates living in multimale-multifemale groups. Engelhardt et al. (2005) and Stumpf and Boesch (2005) reported that females might prefer high-quality (which for primates is often equivalent to high-ranking) males around ovulation, but other males during nonfertile periods. It has been observed that some females of primate species choose to mate with subordinate males (*Macaca mulatta*: Manson, 1992, 1994; *Macaca fuscata*: Huffman, 1991; Soltis et al., 2001). By mating with several males, females may confuse paternity, thus reducing the incentive for males to commit infanticide (Hrdy, 1979). Borries et al. (1999), Crockett and Sekulic (1984), Deag (1980) and van Schaik (1994) suggested that paternity confusion may increase the likelihood that males will provide direct benefits to females or their offspring, such as infant carrying, tolerance during feeding, and protection from infanticide and/or predation. Arlet et al. (2007) reported that primate females mate with lower ranking males despite efforts of the high-ranking males possibly to avoid inbreeding or as a tactic to avoid their aggression towards juveniles. (Dunbar, 1984; Ohsawa et al., 1993; Berard et al., 1994; Manson 1996) observed evidence that low-ranking and nongroup males use alternative tactics such as sneak copulations to obtain mating with females.

For the goals of species identification in animals, the cytochrome c oxidase subunit 1 (cox1) has been introduced as standard marker. Cox1 could serve as a fast and accurate marker for the identification of animal species, and for the discovery of new species across the tree of life (Hebert et al., 2003). One of the key features of the DNA barcoding project, as proposed by Hebert et al. (2003), is the designation of a single mtDNA fragment at the 50 end of cytochrome coxidase I (COI) gene to act as a 'barcode' to identify and delineate all animal life. A DNA barcode is a short sequence of nucleotides taken from an appropriate part of an organism's genome that is used to identify it at species level. Intraspecific variation in this gene is generally <10% of that observed between species. Moreover, insertions and deletions are rare (Blaxter, 2004). DNA barcoding was employed to resolve species within narrow taxonomic groupings in some of the studies (Brown et al., 2003; Hebert et.al., 2004; Hogg and Hebert, 2004; Paquin and Hedin, 2004; Penton et al., 2004; Whiteman et al., 2004; Barrett and Hebert, 2005; Greenstone et al., 2005; Lambert et al., 2005; Vences et al., 2005; Ward et al., 2005; Hajibabaei et al., 2006; Smith et al., 2006b) or to identify higher taxa from wider assemblages of animals in others (Hebert et al., 2003; Remigio and Hebert, 2003; Barrett and Hebert, 2005). In addition, DNA-sequencing technology has been used for identifying organisms from other Kingdoms including plants (Kress et al., 2005; Shaw et al., 2005), bacteria (Woese, 1987; Unwin and Maiden, 2003), protists (Cavalier, 2004) and viruses (Mayo and Horzinek, 1998). Lorenz et al. (2005) generated molecular bar code for 56 species of primates using 'universal cox 1 primers' and primers developed specifically for the primate taxa. Of the 56 species, only *Macaca mulatta* is included among the eight species that are found in Mizoram.

CHAPTER III

AIMS AND OBJECTIVES

Distribution of northern pigtailed macaque species in India is very limited. The population status of this species is not well known. Much work has not been done so far on the social and breeding behaviours of northern pigtailed macaques in India. Zoos provide hybrid environment to animals. This study was undertaken to understand the behaviour of northern pigtailed macaque and its nutritional requirement that is necessary for the development of management strategies and growth of animals under captivity. The following aims were set forth to be carried out in this study.

1. To study the food preference and the profile of gastrointestinal parasites.
2. To study the social behaviour and gestural communications.
3. To study the breeding and reproductive behaviour.
4. Molecular characterization through DNA barcode.

CHAPTER IV

STUDY SITE AND SUBJECTS

Study site

Mizoram lies between 21^{o} 56’N – 24^{o} 31’N latitudes and 92^{0}16’E – 93^{0} 26’ E longitudes. It has a total geographical area of 21,081 square kilometers. The physical set-up of Mizoram is composed predominantly of mountainous terrain of tertiary rocks. The elevation ranges from 40 m to 2157 m. Mizoram enjoys a moderate climate owing to its tropical location, with minimum temperature of 11^{o} C in winter and maximum of 31^{o} C in summer. The entire state is under the direct influence of monsoon with the average rainfall of 250 cm per annum. Out of the total geographical area, about 75% is covered by vegetation. The type of vegetation ranges from tropical trees to sub-tropical trees. It consists of three forest types: Tropical Wet-Evergreen Forest, Tropical Semi-Evergreen Forest and Mountain Sub-Tropical forest (Pachuau, 1994).

The study was conducted on captive northern pigtailed macaque (*Macaca leonina*) housed at Aizawl Zoological Park, Aizawl. Aizawl Zoological Park was established in 2002, it covers an area of 65 hectare and is situated 14 km away from Aizawl, the state capital of Mizoram. The Zoo is located at 23.47”41-23.47”23 latitude N and 92.40”00-92.40”05 longitude E. Seven species of primates viz; rhesus macaque (*Macaca mullata),* assamese macaque *(Macaca assamensis),* stumptailed macaque *(Macaca arctoides),* northern pigtailed macaque *(Macaca leonina),* western hoolock gibbon (*Hoolock hoolock*), capped langur (*Trachypithecus pileatus)* and slow loris (*Nycticebus bengalensis)* are maintained under captivity.

Subjects

The study group of captive northern pigtailed macaque (*Macaca leonina*) consisted of 12 individuals (3 adult males, 4 adult females, 3 immature males and 2 immature females). They are housed in an open enclosure of 850m^2 with two adjacent indoor rooms which can be easily closed and opened using sliding doors. Inside the enclosure, all the animals could be readily observed at all times. A linear dominance hierarchy exists among the males. However, dominance hierarchy was not observed among the adult females. All the individuals in the study were identified on the basis of different morphological features such as face colour, genital colour, size of the body, size and shapes of the nipples (in case of females), pattern of scars or patches on face or the body. Different aspects of the study were done from January, 2016 to March, 2019.

Table 1: Taxonomic position of *Macaca leonina*

Order	**:**	Primates
Sub-order	**:**	Haplorrhini
Infra-order	**:**	Simiiformes
Super-family	**:**	Cercopithecoidea
Family	**:**	Cercopithecidae
Sub-family	**:**	Cercopithecinae
Genus	**:**	*Macaca*
Species	**:**	*leonina*

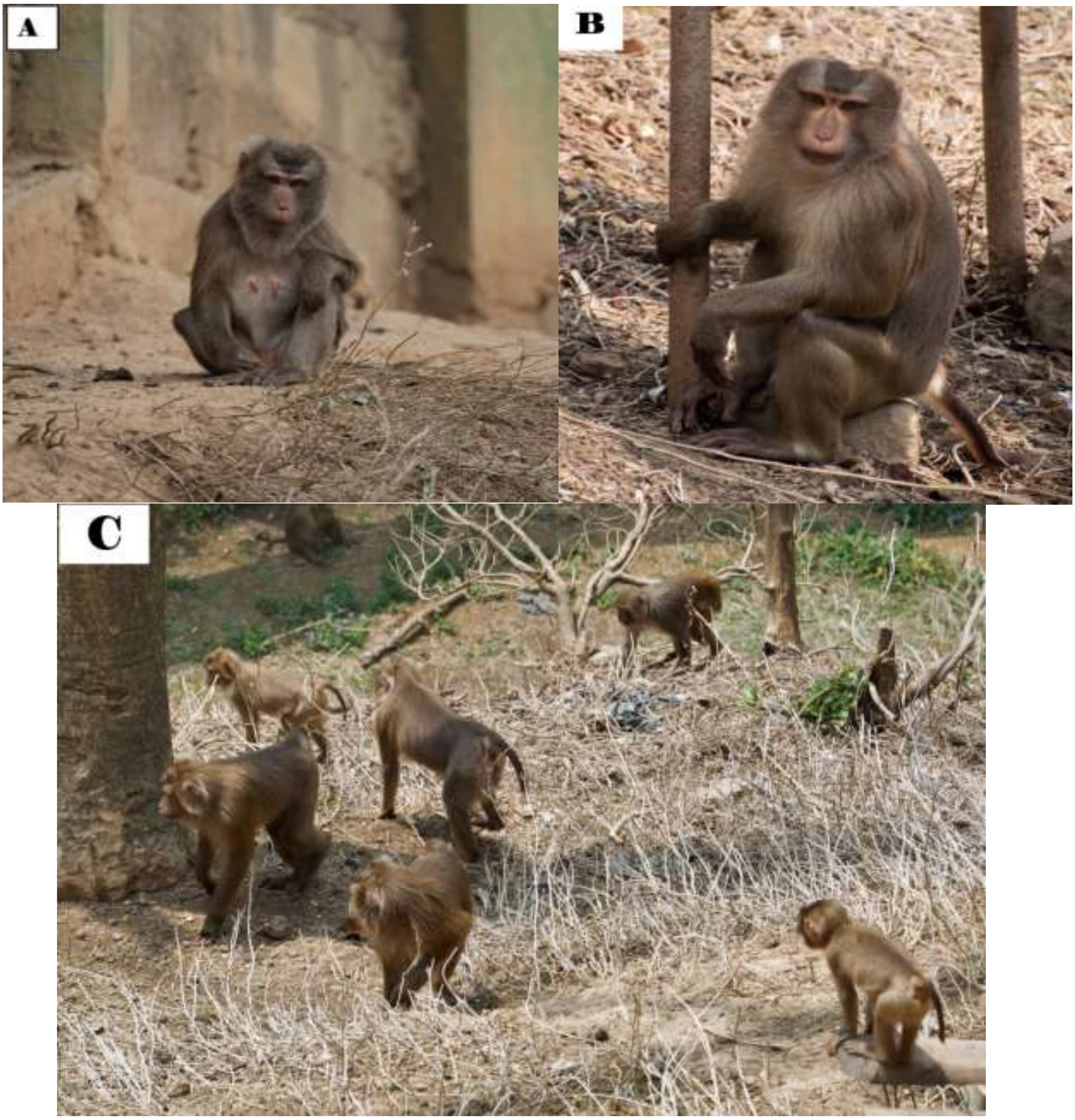


Photo plate 1: Northern pigtailed macaques at Aizawl Zoo

A: Adult female

B: Adult male

C: Group of study animals

CHAPTER V

FOOD PREFERENCE

INTRODUCTION

The majority of primate species feed on a large variety of plant foods (Richard, 1985). Most primate species, being selective feeders, spent a considerate proportion of their time searching for preferred foods (Hughes, 1993). Field studies have shown that primates do not feed on plant parts randomly, but instead display marked preferences (Chivers, 1998). Underpinning all aspects of nutritional ecology is the need for individuals to procure appropriate quantities of certain macronutrients and micronutrients from the habitat. The nutrient requirement is not uniform among different species or individuals but may vary depending on factors such as body size, metabolic requirements, lifestyle, and digestive system (Parra, 1978; Milton, 1993). It is commonly agreed that the food choices of primates can mainly be attributed to two principal factors: (a) the nutritional and/or the toxic content of the particular plant part (Rusterholz, 1984; Barton and Whiten, 1994; Forbes, 1995; van Wiesen, 1996), and (b) its relative spatial and temporal availability (Belovsky, 1990; Belovsky and Schmitz, 1991, 1994; Castellanos and Chanin, 1996). Primates are able to asses food quality through the sensory information that comes from the food. Senses can help primates to make efficient decisions about food; for example, taste provides an immediate and powerful feedback used to select foods (Dominy et al., 2001). Primates are sensitive to bitter and/or astringent tastes that are associated with the presence of plant secondary compounds, such as alkaloids and glycosides. The latter substances can cause illnesses or even have lethal effects if they are ingested excessively so their taste can function as a cue to inhibit ingestion (Ueno, 2001). Soluble sugars, which are a very important energy source for primates, are also readily perceived and absorbed by the organism (Glaser, 1993). Food preferences involve interactions between taste and the consequences of food ingestion (Provenza, 1996). It has been hypothesized that frugivorous primates use sweetness as a criterion for food choice (Laska et al., 1996, 1998). Optimal foraging theory predicts that natural selection will favor individuals that maximize their rate of net intake of energy or other critical nutrients and that food selection patterns observed are thus adaptive (Stephens and Krebs, 1986). This study aimed at assessing the preference of the provisioned food items given to the captive group of northern pigtailed macaque at Aizawl Zoological Park, and if the preference correlates with the amount of certain nutrients present in these food items.

MATERIALS AND METHODS

Experimental animal

The experiment was conducted with 10 northern pigtailed macaques (*Macaca leonina*) housed at Aizawl Zoological Park. The amount of food offered daily to the pigtailed macaques was such that leftovers were still present on the floor the next morning, and thus, it is unlikely that ravenous appetite affected their ingestive behaviour during the tests.

Experimental layout

The food preferences of the animals were assessed using a two-alternative choice test method (Laska et al., 2000a). The animals were presented with pairs of food items and their choice behaviour i.e. which of the two food items they consumed first was recorded. The animals were tested singly to identify their choices and also to avoid interference by other individuals. The animals were separated for 3 sessions each day at 9:00a.m., 12:00p.m. and 3:00p.m. The specific feeding schedule was chosen to account for possible diurnal changes in food preference. On separation, the animals were allowed to choose between a pair of simultaneously presented pieces of food. All the food items were cut into cubes of similar size to facilitate easy handling of food and to ensure that size differences would not affect their selection. The food items were on a rectangular dish of 30 x 20 cm. In order to understand the preference and to prevent the animals from taking both food items at the same time, the food items were placed more than 30 cm apart. In order to minimize the inevitable intra specific variations in nutrient composition, food items of a given type with the same degree of ripeness were always presented during the experiment. In order to counterbalance the possible positional preferences, the position of the food items was exchanged after the first presentation. For example, banana is placed to the left and tomato to the right and their positions are exchanged i.e., tomato to the left and banana to the right. If the same food item is chosen under both situations, the food is recorded as the preferred one. In certain cases when the food choice is not same on the two sites, a third site is chosen to confirm the food choice.

Each animal was presented with all the 28 possible binary combinations of the 8 types of food for a total of 10 times. Food samples used in the test includes banana, papaya, apple, carrot, mustard, pumpkin, tomato, chayote. All of these foods are part of the monkeys' diet in captivity and thus are familiar to them and readily taken and consumed when presented singly. In order to minimize the inevitable intraspecific variations in nutrient composition, food items with the same degree of ripeness was always presented.

Preference criterion

A total of 2800 choices (28 binary combinations X 10 presentations per animal X 10 subjects) was recorded. The food preference rankings were established via the following 3 criteria:

Criterion 1 (group level): If a given food type in a binary combination is consumed ≥6 times by ≥9 individuals, the food item was given 2 points. If the food item is consumed ≥6 times by <9 individuals, the food is given 1 point. Therefore, the theoretical maximum score for a food in this criterion is 14 points: 7 combinations X 2 points.

Criterion 2 (individual level): For each animal that consumed a food as a prime in the majority of trials with a given binary combination, i.e. in ≥ 6 out of 10 presentations, we gave it 1 point. If a subject chose both alternatives in a given binary combination 5 times each, then we gave each of the food item 0.5 points. The theoretical maximum score for any type of food with this criterion is 70 points: 7 combinations X 10 points.

Criterion 3: The sum total of choices was built for each of the 8 food items across all the binary combinations. The theoretical maximum score for any type of food with this criterion is 700 points: 7 combinations X 10 subjects X 10 decisions per individual.

Data analysis

Nutrient contents of the food items were assessed for proximate analysis as per A.O.A.C. (1975). 2-tailed binomial tests were performed using the sum total of choices for each member of a given binary combination to determine significant preferences at the individual level and group level. The correlations between the food preference ranking and the nutrient contents therein was evaluated by calculating Spearman rank-order correlation coefficients (r_s). All the statistical analyses were done with SPSS ver.18.0

RESULTS

Food preferences

Table 2 shows the food preference rankings derived from the 2800 choices made by the monkeys according to the three criteria. Banana was the most preferred food item, followed by papaya, apple, carrot, mustard, pumpkin, tomato and chayote is the least preferred food in the 3 criteria. The food preference ranking is similar in all the 3 criteria. This phenomenon shows that food preference among the provisional food items clearly exist in pigtailed macaque under captivity. Table 3 represents the group scores, i.e. the number of choices for each member of a given pair of food summed across the 10 animals in the 28 binary combinations.

Table 3 indicates the number of choices (from n = 10 individuals) for each member of a given pair of food items. The first value applies to the food item to the left and the second value applies to the food item on the top. ← indicates a significant preference for the food item to the left ($p < 0.05$). With all of the 28 possible binary combinations, the subjects displayed a statistically significant preference for one of the options (2-tailed binomial test, p<0.05). Banana is clearly the most preferred food item and is significantly preferred over all the options (binomial test, p <0.05) for all the 7 combinations. Chayote is clearly the least preferred food item.

Table 2: Food items and their corresponding rank order of preference in Northern Pigtailed Macaque

CRITERION 1		**CRITERION 2**		**CRITERION 3**	
Food items	**Score**	**Food Items**	**Score**	**Food Items**	**Score**
1. Banana	13	1. Banana	68	1. Banana	596 (85.14%)
2. Papaya	11	2. Papaya	58.5	2. Papaya	538 (76.85%)
3. Apple	10	3. Apple	51.5	3. Apple	473 (67.57%)
4. Carrot	9	4. Carrot	40.5	4. Carrot	414 (59.14%)
5. Mustard	6	5. Mustard	29.5	5. Mustard	328 (46.85%)
6. Pumpkin	5	6. Pumpkin	20.5	6. Pumpkin	234 (33.42%)
7. Tomato	2	7. Tomato	10	7. Tomato	156 (22.28%)
8. Chayote	1	8. Chayote	1	8. Chayote	45 (6.42%)

Table 3: Choice behaviour of the subjects in the food preference tests

	Banana	**Papaya**	**Apple**	**Carrot**	**Mustard**	**Pumpkin**	**Tomato**	**Chayote**
Banana	**X**	←	←	←	←	←	←	←
Papaya	31:69	**X**	←	←	←	←	←	←
Apple	16:84	29:71	**X**	←	←	←	←	←
Carrot	21:79	21:79	27:73	**X**	←	←	←	←
Mustard	13:87	14:86	16:84	19:81	**X**	←	←	←
Pumpkin	9:91	16:84	12:88	18:82	17:83	**X**	←	←
Tomato	9:91	14:86	8:92	14:86	12:88	17:83	**X**	←
Chayote	1:99	1:99	2:98	3:97	3:97	10:90	25:75	**X**

Correlational analysis

Estimation of the amount of moisture content, ash content, protein content, lipid content and carbohydrate contents were done as per A.O.A.C. (1975). Table 4 shows the proximate macronutrient contents of the food items. Table 5 summarizes the Spearman rank-order correlation between food preference ranking and nutrient contents. The food preference ranking is significantly positively correlated with the total carbohydrate content (r_s=0.929, p=0.001*) i.e. the pigtailed macaques clearly preferred foods that are high in total carbohydrate contents over foods that are low in total carbohydrate content. No other significant correlation between the food preference ranking and the content of any other macronutrient or micronutrient was found in this study.

Table 4: Macronutrient contents of the food items

Food items	Nutrient content (per 100g)				
	Moisture	Ash	Protein	Lipid	Carbohydrate
Banana	70.21%	29.79g	1.12g	0.27g	24.21g
Papaya	84.50%	15.5g	0.35g	0.28g	12.22g
Apple	82.16%	17.84g	0.22g	0.21g	12.81g
Carrot	76.29%	23.71g	0.81g	0.23g	10.12g
Mustard	91.28%	8.72g	2.32g	0.51g	6.45g
Pumpkin	89.56%	10.44g	1.22g	0.17g	6.42g
Tomato	95.29%	4.71g	0.91g	0.18g	4.5g
Chayote	92.55%	7.45g	0.75g	0.11g	3.91g

Table 5: Correlational analysis between the amount of nutrients in food items and the food preference displayed by the animals

Nutrients	r_s	p value
Moisture	0.690	0.058
Protein	-0.190	0.651
Lipid	0.690	0.058
Carbohydrate	0.976	0.001*
Ash	-0.405	0.320

r_s= Spearman correlation value, p= Probability value

DISCUSSIONS

Information on the food preference and the nutritional requirement of a species is necessary for the conservation and maintenance in captivity. This study demonstrated that captive northern pigtailed macaques displayed marked preferences in a 2-alternative choice situation and that the preferences are significantly positively correlated with the total carbohydrate content of the food items. In addition, the monkeys' food preferences were stable across the day and was very similar between the individuals tested.

It is well established that several factors other than the nutritional value of a potential food item may affect an animal's choice. In a 2-alternative test situation, nonhuman primates usually, though not necessarily, prefer the larger size of fruits (Menzel and Draper, 1965). This potential bias was controlled by presenting pieces of food of the same size. Side preferences may also affect a subject's choice behaviour (Jildmalm et al., 2008) and this possibility was controlled by presenting the same pair of food items twice or thrice with change of sides.

Differences in palatability and/or in novelty of the food items might also affect choice behaviour (Fragaszy et al., 1997). To control for the factors, it was taken care that all the type of food used were familiar to the animals and preliminary tests performed showed that all the types of food were readily taken by all the subjects when presented singly, suggesting that none of them were unacceptable. The feeding regimen employed ensured that neither ravenous hunger nor satiety affected the preference.

Social contexts also affect food choice in primates (Visalberghi et al., 1998), which was controlled by testing the subjects individually. In the wild, white-handed gibbons (Raemaekers, 1978) and several other primate species (Thorington, 1970; Whitten, 1982) vary in both quantity and type of consumed food with the time of day. To control for possible biases from diurnal variation in diet, the 10 presentations of a given binary combination of food items was spread as evenly as possible across the sessions at 9:00a.m., 12:00p.m. and 3:00p.m. Thus, it can be believed that food preferences indeed reflect the pigtailed macaque’s ability to choose between foods on the basis of perceived differences in nutritional value.

Northern pigtailed macaques displayed marked preference towards foods that are high in carbohydrate content. This finding is in accordance with various other findings in which primates prefer foods that are high in carbohydrate content (pigtailed macaques: Laska, 2001; white handed gibbons: Jildmalm et al., 2008; spider monkeys: Laska et al., 2000a, 2000b; gorillas and chimpanzees: Remis, 2002). However, Laska et al. (2001, 2003) observed a positive correlation between the food preferences displayed by captive squirrel monkeys (*Saimiri sciureus*), and pacas (*Agouti paca)* and total energy content in the foods. Lalremruati et al. (2017, 2018) also observed positive correlation between the food preference ranking of stumptailed macaque and the lipid and protein content of the foods. Pigtailed macaques and white handed gibbons include a high proportion of carbohydrate rich foods in their natural diet (Bollard, 1970; Chivers, 1984; Simmen et al.,, 1999). Therefore, total carbohydrate content in foods might be an important determinant of food choice for the species (Racemaekers, 1978; Richard, 1985; Ungar, 1995).

Soluble carbohydrates have been reported to influence food choice by a wide variety of vertebrate frugivores, including birds, bats and primates (Wrangham et al., 1991; Leigh, 1994; Herrera, 1999). Carbohydrates are the main source of energy that is ingested by the human body (Caffall et al., 2009; Asif et al., 2011). The primary role of carbohydrate is to provide energy to all the cells in the body and dietary fiber. Carbohydrates also play an important role in the structure and function of the body organs and nerve cells (Asif et al., 2011). The brain needs to use glucose as an energy source, since it cannot use fat for this purpose. It is for this reason that the level of glucose in the blood must be constantly maintained above the minimum level. (Asif et al., 2011). Therefore, it can be argued that the captive group of northern pigtailed macaque prefers foods which are high in carbohydrates, as carbohydrates is constantly needed for the proper functioning of the body.

CHAPTER VI

PROFILE OF GASTROINTESTINAL PARASITES

INTRODUCTION

Non-human primates are susceptible to a variety of diseases due to parasites, both in the wild and in captivity (Kuntz 1982). The parasites and infectious diseases have become a major concern for survival and conservation of animals as they can lead to physical and physiological inefficiencies and ultimately to mortality, dramatic population decline, and even local extinction if proper remedial measure is not initiated on time (Smith et al., 2006a; Levecke et al., 2007; Wisely et al., 2008; Smith et al., 2009; Aguirre et al., 2017). Parasites can influence host survival and reproduction both directly through pathological effects and indirectly by reducing the host's physical condition (Boyce, 1990; Hudson, 1992; van Vuren 1996; Glaser and Kiecolt-Glaser 2005; Coe 2011; Hilser et al., 2014). Gastrointestinal parasites in animals in captivity include zoonotic species to humans and raise public health concerns (Levecke et al., 2007; Olayide and Adekunle, 2008; Ajibade et al., 2010; Akainboye et al., 2010; Opara et al., 2010; Otegbade et al., 2014). Captive animals are supposed to have low prevalence as anthelmintic measures are practiced, however infection maybe more due to unhygienic conditions of cages and surroundings. Crowding of animals in cage, type of food and feeding practices are key factors in the development of endoparasites in zoo animals (Malan et al., 1997; Mul et al., 2007; Sanchez et al., 2009). Nutrients in the food consumed by the host also affects its susceptibility to infection (Moore, 2002; Roberts and Janovy, 2008), host density has been shown to have a major impact on the prevalence in primates (Arneberg, 2002; Mbora and McPeek, 2009).

Inadequate information on diseases and parasite of zoo animals is a major limiting factor in the management of zoos. Investigations into prevalence, distribution and biology of parasites of zoo animals are important for planning and control of parasites. Hence, there is a need for a regular program for gastrointestinal parasite surveillance and measures for control based on correct diagnosis, effective treatment and proper prophylaxis to ensure sound health of zoo animals (Ajibade et al., 2010; Moudgil et al., 2014). Knowledge about the parasite prevalence and their intensity in primates will help the zoo managers for proper management of primates as well as to keep up the fitness and survival chances. This study attempts to understand the parasitic profile of this threatened primate species, northern pigtailed macaque (*Macaca leonina*) so that the zoo authorities may undertake appropriate measures for prevention of parasitic infection for this species and also to primates in general.

MATERIALS AND METHODS

Faecal Sample Collection

Fresh stool samples were collected from 12 individuals (7 males and 5 females) of the study group for three seasons, summer (March to June, 2017), monsoon (July to September, 2017) and winter (October, 2017 to February, 2018). Samples were examined macroscopically for the presence of larval or adult of various parasites at different stages. Samples were collected in the morning hours (6:00 a.m. to 9:00 a.m.) and were stored in 10% formalin at sampling site for further processing. The collection tubes were shaken vigorously to maximize contact between sample and storage solution. For the control of parasitic infection, Piperazine hydrate liquid 61% by volume has been given to the animals at the intervals of once in three months by the zoo authorities.

Sample Processing

Three methods as recommended by Gillespie (2006) were used for the identification of parasitic infection, i.e., direct smear, faecal floatation and faecal sedimentation.

Direct Smear: A thin smear of faecal material with normal saline was prepared on a microscope slide and observed under microscope.

Faecal floatation*:* Approximately 1g of faeces taken was placed into a 15 ml centrifuge tube. The tube was filled 2/3 of with de-ionised water and homogenized with a wooden spatula, then centrifuged for 10 minutes at 1800 rpm. The supernatant was decanted and the faeces was re-suspented in sodium nitrate ($NaNO_{3)}$ solution. The tube was filled to the meniscus with $NaNO_3$ and a cover slip was placed on the mouth of the tube and left for 10 minutes. The cover slip was removed and placed on a labelled slide. Single slide for each individual sample were systematically scanned using a microscope.

Faecal sedimentation: 1 g of the preserved faecal sample was put in a centrifuge tube, thoroughly homogenised, topped up and thoroughly mixed with 7ml of 10% formal saline solution which also served as the fixative. The resulting suspension was strained into a clean centrifuge tube using a fine sieve to remove debris. Three millilitres of diethyl ether was then added. The mixture was stoppered and mixed. The mixture was then centrifuged for 3 minutes at 2000 rpm. Debris and fat which formed a floating plug were dislodged using an applicator stick and the supernatant was discarded. Using a Pasteur pipette, a drop of each of the remaining sediment was transferred to a clean glass microscope slide to make a wet smear. Lugol's iodine solution (0.15%) was used to stain the slide. Sediments were further screened and analyzed for identification of parasites and their different stages. Kruskal-Wallis tests were used to assess the variation on the prevalence of each gastrointestinal parasite between the three seasons. Statistical analysis was carried out with SPSS version 18.0.

RESULTS

The study animals were found to be infected with two major groups of parasites: protozoa and nematodes. Four species of parasites, namely, *Balantidium coli,* a protozoa; and Strongyle, roundworm (*Ascaris lumbricoides),* and whipworm (*Trichiuris trichiura)*, the nematodes, were recorded. Photos of the ova of all species recorded are given on Photo plate 2. Out of 110 samples were analyzed during the study, 68 samples were positive with gastrointestinal parasites. Therefore, the rate of infection with gastrointestinal parasites was 61.82%. Seasonal variations in the rate of infestation and different parasite species recorded also varied.

Summer season

In the summer season, the prevalence of parasites was 52.94%. In this season, protozoan infection was found to be highest with prevalence/infection rate of 38.23%, and among nematodes, the highest infection rate was with *Trichiuris trichiura* (35.29%) followed by *Strongyle* (26.47%) and *Ascaris lumbricoides* (23.53%).

Monsoon season

The overall prevalence of gastrointestinal parasites during monsoon season was 67.57%. In the monsoon season, the rate of infection with *Balantidium coli,*was highest with 56.75% prevalence, followed by *Strongyle* and *Trichiuris trichiura* (37.83% and 37.83% respectively) and *Ascaris lumbricoides* again with least infection (27.02%).

Winter season

The overall prevalence of parasite during the winter season was 64.11%. Among the parasite species found in this season, the rate of infection with *Trichiuris trichiura* was highest, i.e., 38.46%, followed by *Balantidium coli* (35.89%), *Strongyle* (33.33%) and *Ascaris lumbricoides* (25.64%).

Seasonal variation

The seasonal comparison of prevalence (%) of all four types of parasites is given on figure 1. The overall prevalence of gastrointestinal parasites was highest during monsoon season (67.57%), followed by winter season (64.11%) and least during the summer season (52.94%). Kruskal-Wallis test revealed no significant variation on the overall rate of infection with gastrointestinal parasites during the three seasons (χ^2=4.017, df=2, p=0.132). Moreover, the prevalence of different gastrointestinal parasites found during the study was more or less similar among the three seasons (Table 6.)

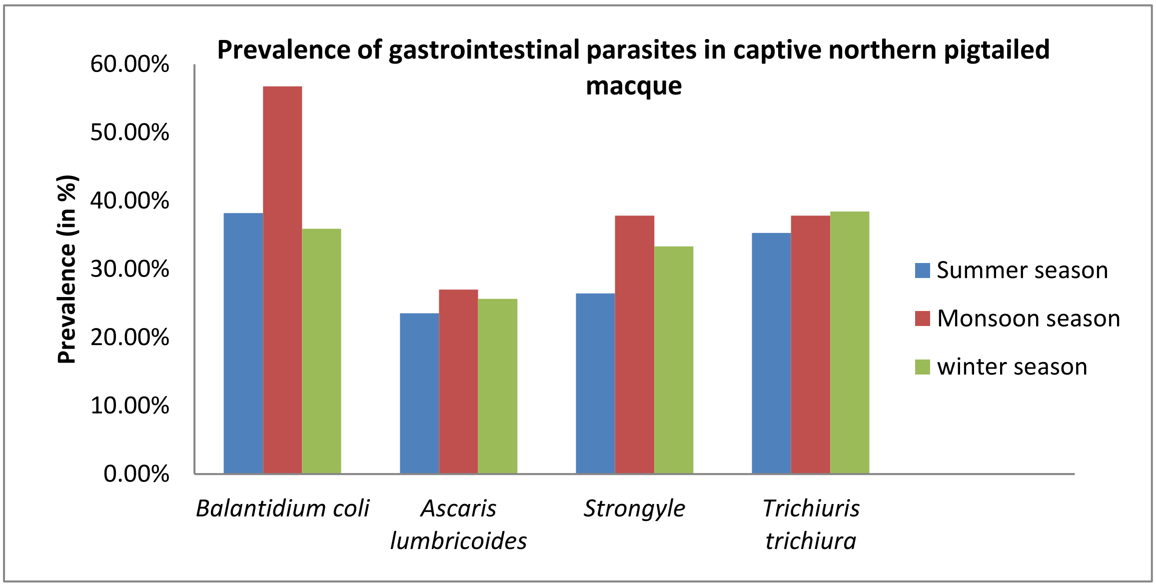


Figure 1: Prevalence of parasites during summer, monsoon and winter season

Table 6: Prevalence of different gastrointestinal parasites across the three seasons

Name of parasite	Prevalence (in %)			χ^2	p value
	Summer season	Monsoon season	Winter season		
Balantidium coli	38.23	56.75	35.89	2.340	0.310
Ascaris lumbricoides	23.53	27.02	25.64	0.057	0.972
Strongyle	26.47	37.83	33.33	0.504	0.777
Trichiuris trichiura	35.29	37.83	38.46	0.366	0.833

χ^2= Chi square value, p= Probability value

DISCUSSIONS

Zoos serve an important role in the conservation and perpetuity of threatened species. In several cases, animals maintained in captivity are the only chance for the species continuation in which the species has been extinct in the wild. Therefore, it is crucial to maintain proper health and well-being of the threatened animals in captivity for their survival and better adaptation to the natural wild conditions, if such needs arise. Several parasitic infections have been reported in non-human primates, both in captivity (Levecke et al., 2007; Cordon et al., 2008; Lim et al., 2008; Nath et al., 2012; Barbosa et al., 2015; Margono et al., 2015) and in the wild (Huffman et al., 1997; Legesse and Erko, 2004; Parr, 2013; Kouassie et al., 2015). The prevalence of gastrointestinal parasites observed in the present study is 61.82%. This is in accordance with the studies conducted by other researchers, such as, Ajibade et al. (2010) and Opara et al. (2010), with prevalence rates of 62.5% and 61.5% respectively in captive animals. Parasitic diseases are reported to be common to zoo animals in tropical countries due to the climatic factors that favour the development of parasites such as light, temperature and humidity (Opara et al., 2010). Protozoa (*Balantidium coli)* and nematodes (*Strongyle, Ascaris lumbricoides,* and *Trichiuris trichiura*) were detected in the present study. These two groups of parasites were also reported in Belgium zoo with more diversity within these groups in prosomian, old world monkeys and new world moneys including some apes (Levecke et al., 2007). The protozoa and nematodes are highly prevalent and are the most diverse groups of gastrointestinal parasites even in wild nonhuman primates (Kouassi et al., 2015). The present study also indicates high prevalence of protozoa (*Balantidium coli*) in both summer and monsoon seasons as compared to nematodes (figure 1), similar to the study conducted by Levecke et al. (2007) on captive primates. Trematodes and cestodes were not detected in this study. This could be due to the fact that these parasites require an intermediate host for their transmission and that is less likely in the captive environment (Atanaskova et al., 2011). The nematodes and some coccidian parasites have a direct life cycle, without any intermediate host and are transmitted by feco-oral route through contaminated feed, water, and soil and have the potential to accumulate in a captive environment (Thawait et al., 2014). Contaminated environment in the zoos could be through contaminated water or fodder, and even zoo workers have also been reported to play a role in transmission by acting as vectors and transmitting parasites through their shoes, clothes, hands, food, or with working tools (Adetunji 2004; Otegbate et al., 2014). Attendants of animal enclosures could act as vehicles for cross transmission. Also, the animals serve as potential reservoirs that could transmit gastrointestinal parasites to zoo keepers and possibly to visitors. Based on this study, it is recommended that upgraded and more effective regular preventive as well as prophylactic measures are needed to be included in the management schedule of these animals at regular interval. Physical and chemical based hygiene are also needed as a part of management programs for captive animals. Zoo attendants or animal enclosure attendants should be given specific training for keeping a proper record of such anthelmintics treatment to animals and to fumigate the enclosure to upkeep the hygiene so that infection can be prevented within and across the enclosure. This study may help the zoo authorities to develop better parasite management plans for northern pigtailed macaque, which may further benefit the breeding programs undertaken for the monkeys, as proper health condition is needed for successful breeding.

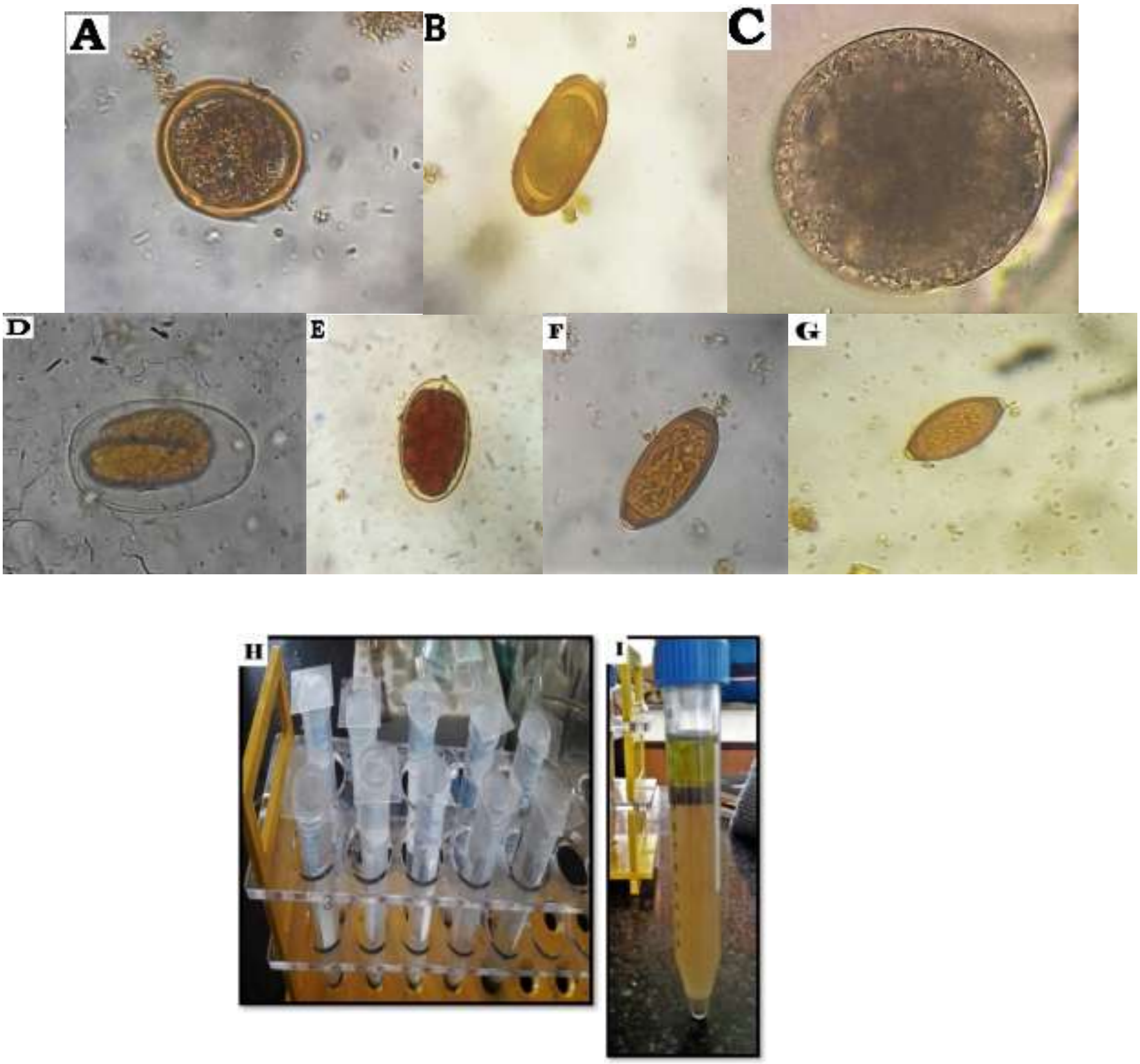


Photo Plate 2: Different gastrointestinal parasites and faecal processing

A and B: Ova of *Ascaris lumbricoides*
C: Cyst of *Balanditium coli*
D and E: Ova of strongyle
F and G: Ova of *Trichiuris trichiura*
H: Faecal floatation
I: Faecal sedimentation

CHAPTER VII

GROOMING BEHAVIOUR

INTRODUCTION

Grooming behaviour encompasses all forms of care and attention to the body surfaces (Saunders, 1988). Grooming refers to the act of treading and manipulation of the body surface (Hutchins and Barash, 1976; Tanaka and Takefushi, 1993; Pe´rez and Vea, 2000). The social functions of grooming include the establishment and maintenance of affiliative relationships and the reduction of tension and aggression between individuals (Terry, 1970; Saunders, 1988; Kimura, 1998; Kutsukake and Clutton-Brock, 2006). In addition to its hygienic functions, allogrooming is generally considered to maintain social bonds between group members (Dunbar, 1988; Henzi and Barett, 1999; Schino and Aureli, 2008). Grooming may also have important indirect or direct health consequences. With respect to indirect consequences, receiving grooming reduces heart rate and is thus presumed to improve physiological wellbeing (Boccia et al., 1989; Aureli et al., 1999); grooming others is correlated with a reduction in the stress hormone cortisol in the groomer (Shutt et al., 2007); participation in grooming (receiving or giving) increases the production of endorphins, a biomarker of increased psychological wellbeing (Keverne et al., 1989).

Grooming also has direct effects on the wellbeing of primates via removal of ectoparasites such as lice, fleas and ticks (Freeland, 1981; Saunders and Hausfater, 1988; Tanaka and Takefushi, 1993; Eckstein and Hart, 2000; Hart, 2000; Zamma, 2002; Kutsukake and Clutton-Brock, 2006).

In primate societies, grooming is an integral part of life (Dunbar, 1988, 1991, 2010; Grueter et al., 2013). It constitutes a major social activity in many species of social mammals including ungulates (Mooring et al., 1996; Hart, 2000; Heitor et al., 2006), rodents (Ferron and Lefebvre, 1982), bats (Wilkinson, 1986). Some primate species devote as much as 20% of their total daytime for grooming (Sparks, 1967; Goosen, 1987; Dunbar, 1991; Lehmann et al., 2007; Schino, 2007; Shutt et al., 2007). Grooming is termed as auto or self-grooming if groomer and groomee are the same individual and allogrooming if groomer and groomee are different animals.

Grooming varies with many factors. In some primates, grooming patterns are highly kin biased, and kinship explains a large fraction of the variance in grooming patterns (Schino, 2001; Chapais and Berman, 2004). Seyfarth (1977) suggested that grooming is directed up a dominance hierarchy (i.e. preferential grooming of high-ranking animals). Other studies have documented increased grooming down the hierarchy (Obrien, 1993; Parr et al., 1997; Lazaro-Perea et al., 2004). These conflicting findings may result from differences in social and ecological contexts, which influence how resources are distributed in a social group. In many primates, participation in grooming bouts differs between the sexes and with life history stage. Several studies have considered grooming as a female-biased behaviour established during the first year of life, with females grooming almost twice as often as males (Simonds, 1974; Young et al., 1982). Females tend to form strong social bonds with other females (Wrangham 1980) and grooming is a major contributor to these social bonds (Silk et al., 2003a, 2003b, 2006, 2010). The age of an individual also plays a role in the amount of grooming received or given. Saunders (1988) showed that majority of the grooming bouts were initiated by adult females. Adult male baboons groomed less often than adult females (Saunders, 1988). Because males can provide important services, such as protection against infanticidal attacks and harassment

(Smuts, 1985; Saunders, 1988; Silk et al., 2003a; Nguyen et al., 2012), females may be motivated to groom males in return for these services. This study was carried out to understand the purpose and pattern of grooming in pigtailed macaques, the difference in pattern of grooming in different age, sex and hierarchy in the group, and to investigate the differences in the grooming behaviour of different age groups of pigtailed macaques.

MATERIALS AND METHODS

Subjects

Grooming behaviour was studied on 12 individuals of northern pigtailed macaque (3 adult males, 4 adult females, 3 juvenile males and 2 juvenile females). Observations were recorded by Focal Sampling Technique (Altmann, 1974).

Data collection

The study was carried out for 15 months (May, 2016 to August, 2017). Observations were done on a 10-minute interval for 11 hours in a day (7:00 a.m. to 6:00 p.m.), and 6 days in a week for all the grooming behaviour, the purposes and the sites of grooming. Grooming sites were divided into visible sites (or visible areas) and non-visible sites (or non-visible areas). Visible site includes front part of upper arms, hand, chest, belly with genital region, feet, ventral part of legs and thigh, tail tip, fore arm. Non-visible site includes head and neck, face, shoulder, back part of upper arm, dorsal area of legs and thigh, back with anal region.

Data analyses

Kruskal-Wallis test was carried out to test for variation on the purpose and sites of grooming on different age groups. Wilcoxon Mann-Whitney test was used for pair-wise variation between different age groups. All the statistical analyses were carried out in SPSS version 18.0.

RESULTS

Autogrooming and Allogrooming

The time spent (percentage) for autogrooming and allogrooming by the study animals is given on figure 2. All the age groups performed allogrooming more than autogrooming. However, females performed allogrooming frequently than the males, and the juvenile males performed allogrooming more frequently than the adult males.

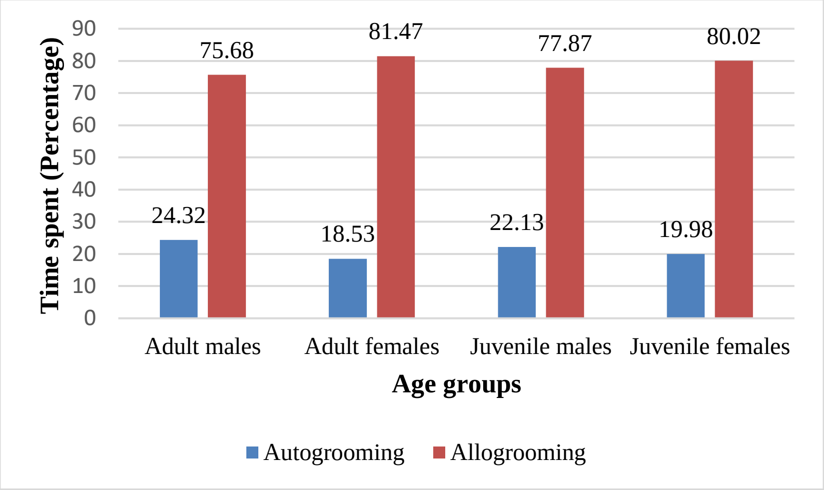


Figure 2: The time spent on autogrooming and allogrooming by different age groups

Purpose of grooming

During the study, four types of grooming purposes were observed, such as, removal of ectoparasites, removal of skin flakes, social function and sexual courtship. The time (%) spent on different purposes of grooming by different age groups are given on table 7. In case of grooming for removal of ectoparasite, the groomer eats the parasite while the skin flakes are thrown away. Grooming for sexual courtship always end with an attempt to mate, therefore, it can be distinguished from grooming for social function. All the age groups of the study animals spent the most time grooming for removal of ectoparasite, followed by social function, sexual courtship and removal of skin flakes. Kruskal-Wallis test was carried out for variation on different categories of grooming purpose in different age groups. Kruskal-Wallis test (table 8) revealed significant variation on the amount of time spent on grooming for social function (χ^2=9.067, df=3, p=0.028*) and sexual courtship (χ^2=9.128, df=3, p=0.028*). There is no significant variation on the amount of time spent on grooming for removal of ectoparasite (χ^2=2.55, df=3, p=0.465) and removal of skin flakes (χ^2=7.34, df=3, p=0.062). The different variations on the purpose of grooming were subjected to pairwise comparison using Mann-Whitney test (Table 9). Mann-Whitney test revealed that adult females spent more time grooming for social function than the adult males ($p<0.05$), and adult males spent more time grooming for sexual courtship than the juvenile males ($p<0.05$). No other variations were observed among the different age groups.

Table 7: Time spent (%) on different purposes of grooming by different age groups of northern pigtailed macaque

Purposes	**Time spent (%)**			
	Adult males	**Adult females**	**Juvenile males**	**Juvenile females**
Removal of ectoparasite	47.38	48.55	45.45	48.74
Skin flakes	7.99	6.811	9.11	8.61
Social function	26.31	31.74	28.82	29.03
Sexual courtship	18.31	12.89	16.63	13.62

Table 8: Kruskal-Wallis test for grooming purpose among different age groups

Purposes	χ^2	**p value**
Removal of ectoparasite	2.55	0.465
Skin flakes	7.34	0.062
Social function	9.067	0.028*
Sexual courtship	9.128	0.028*

χ^2=Chi square value, p=Probability value

Table 9: Mann-Whitney pairwise test for variation on the different grooming purposes among different age groups

Age groups	Social function			Sexual courtship		
	U	**W**	**p**	**U**	**W**	**p**
Adult males vs adult females	0.000	10.000	0.034*	3.500	9.500	0.368
Adult males vs juvenile males	1.000	7.000	0.077	0.000	6.000	0.028*
Adult males vs juvenile females	0.000	3.000	0.064	0.000	3.000	0.060
Adult females vs juvenile males	1.000	4.000	0.165	1.000	4.000	0.140
Adult females vs juvenile females	0.000	1.000	0.157	0.000	1.000	0.157
Juvenile males vs juvenile females	1.500	2.500	0.717	0.000	1.000	0.114

U=Mann-Whitney U, W=Wilcoxon W, p= Probability value

Site of grooming

Visible area

The time (%) spent on grooming visible areas by different age groups are given on table 10. Adult females spent the most time grooming the visible sites, followed by juvenile females, juvenile males and adult males. Kruskal-Wallis test revealed significant variation on the amount of time spent on grooming the visible areas by different age groups (χ^2=9.755, p=0.021*). These variations were subjected to pairwise test using Mann-Whitney test (Table 11). Mann-Whitney test revealed that adult females spent significantly more time grooming the visible areas than the adult males and the juvenile males ($p<0.05$). No other significant variation exists among the other age groups.

Table 10: Time spent on grooming visible sites by different age groups

Age groups	Time spent on grooming visible sites (%)
Adult males	19.59
Adult females	32.43
Juvenile males	20.94
Juvenile females	27.02

Table 11: Mann-Whitney pairwise test for grooming different areas of visible sites

Age groups	U	W	p
Adult males vs adult females	288.5	753.5	0.017*
Adult males vs juvenile females	447.5	909	0.929
Adult males vs juvenile females	444	912.5	0.97
Adult females vs juvenile males	279	744	0.011*
Adult females vs juvenile females	2	8	0.274
Juvenile males vs juvenile females	14	42.5	0.2

U=Mann-Whitney U, W=Wilcoxon W, p= Probability value

Non-visible area

The time (%) spent on grooming non-visible area by different age groups of northern pigtailed macaque is given in table 12. Adult females spent the most time grooming non-visible sites, followed by juvenile females, juvenile males and adult males. Kruskal-Wallis test revealed significant variation for the time spent on grooming non-visible sites ($\chi2$=9.311, df=3, p=0.025*). These variations were subjected to Mann-Whitney pairwise comparison (Table 13). Mann-Whitney test shows that adult males spent less time grooming the non-visible sites than the other age groups ($p<0.05$). Adult females spent more time grooming the non-visible sites than juvenile males and juvenile females ($p<0.05$). No significant variation exists on the time spent for grooming non-visible sites by the juvenile males and juvenile females.

Table 12: Time spent on grooming non-visible area by different age groups

Age groups	**Time spent on grooming non-visible sites (%)**
Adult males	15.08
Adult females	41.09
Juvenile males	20.68
Juvenile females	23.13

Table 13: Pairwise test of the time spent on grooming different non-visible sites by different age groups

Age Groups	**U**	**W**	**p**
Adult males vs Adult females	4.500	32.500	0.010*
Adult males vs Juvenile males	0.000	6.000	0.034*
Adult males vs Juvenile females	5.000	33.000	0.012*
Adult females vs Juvenile males	0.500	6.500	0.048*
Adult females vs Juvenile males	5.000	33.000	0.012*
Juvenile males vs Juvenile females	13.000	41.000	0.140

U=Mann-Whitney U, W=Wilcoxon W, p= Probability value

Visible and non-visible area

More time was spent for grooming non-visible sites than the visible sites by all the age groups. A graphical representation of the time spent on grooming the visible sites and non-visible sites by different age groups is given on figure 3. Mann-Whitney test revealed significant variation on the time spent on grooming visible sites and non-visible sites by the study animals (U=1983.000, W=6639.000, p=0.001*). Mann-Whitney test for each age group for grooming visible and non-visible sites is given on table 14.

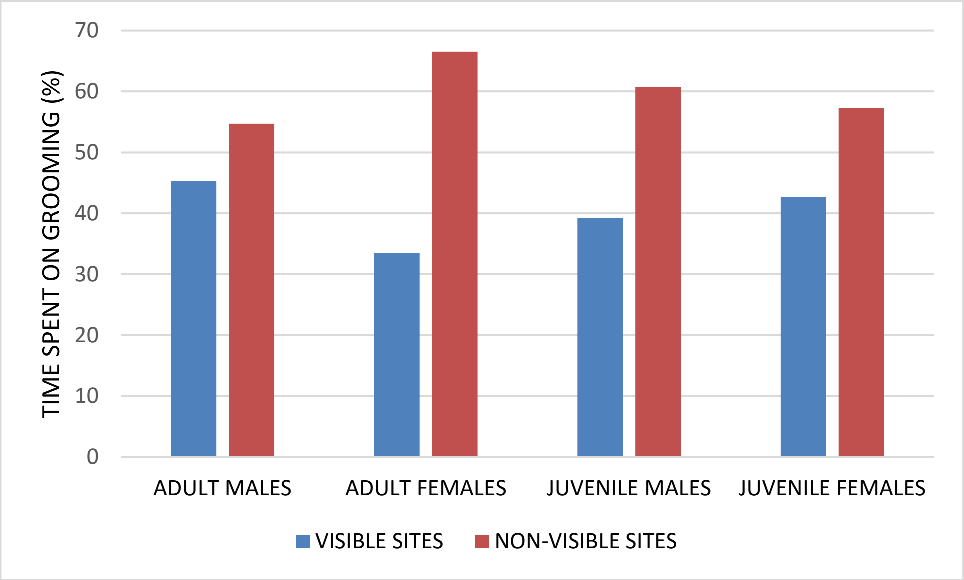


Figure 3: Time spent on grooming visible and non-visible region by different age groups

Table 14: Mann-Whitney test for time spent on grooming visible and non-visible sites by different age groups

Age groups	**U**	**W**	**p**
Adult males	196.500	724.500	0.001*
Adult females	128.500	428.500	0.005*
Juvenile males	106.000	406.500	0.001*
Juvenile females	57.500	193.500	0.023*

U=Mann-Whitney U, W=Wilcoxon W, p= Probability value

DISSCUSSIONS

Northern pigtailed macaques spent a considerable amount of time on grooming. The purposes and site preference of grooming are almost the same among the different age groups of northern pigtailed macaque. The different age groups perform grooming mainly for their hygienic needs and social function. This is in accordance with other studies which suggest that grooming is assumed to satisfy an individual's hygienic needs such as removal of ectoparasite, skin flakes and debris (Hutchins and Barash, 1976; Freeland, 1981; Barton, 1985; Saunders and Hausfater, 1988; Tanaka and Takefushi, 1993; Eckstein and Hart, 2000; Hart, 2000; Zamma, 2002; Kutsukake and Clutton-Brock, 2006) and to promote social bonding and integration (Carpenter, 1942; Sade, 1965; Terry 1970; Kurlan, 1977; Boccia, 1983; Dunbar, 1988; Kimura, 1998; Saunders, 1988; Henzi and Barett, 1999; Kutsukake and Clutton-Brock, 2006; Schino and Aureli, 2008). The study revealed that adult females spent significantly more time grooming for social function ($p<0.05$) than the adult males. This finding shows that adult males are less involved in the social interactions in the group than the adult females.

The previous studies show that the time spent on grooming is reflected by sexes (Drickamer, 1976; Jones, 1979; Michael and Herberth, 1963; Mehlman and Chapias, 1988), age (Lindburg, 1973), and social interaction. In the present study, adult females spent the most time grooming among all the age groups, followed by juvenile females, juvenile males and adult males. The finding of the present study is similar with these previous findings. Sarkar and Bhattacharya (2015), on their study in captive group of assamese macaques, indicated that adult females spent more time on grooming interaction than adult males during different seasons throughout the year. Likewise, juvenile females also spent more time for grooming than juvenile males. Adult females of captive brown capuchins also spent more time grooming than the other age groups (Parr et al., 1997). Ahumadda (1992) reported on spider monkeys (*Ateles geoffroyi*) that adult females groomed most frequently. However, contrastingly, Brockett et al. (2000) reported that adult males and juveniles were the most frequent groomers and adult females were most frequently groomed.

Previous studies have provided evidence that grooming is immediately reciprocated during grooming interactions, grooming partners tend to give as much grooming as they get (Goosen, 1987; Manson et al., 2004). In this study, the adult females groom others most frequently and in return she gets more grooming from others, as compared to juveniles and adult male. On the other hand, the adult males received less grooming from others. This may be due to less reciprocated grooming and poorly interacting socially with others. Since males holds all the resources, it is the females who must maintain a good relationship with the high ranking males in order to access the food resource. Since females play a major role in maintaining social bonding, they spent more time on grooming interaction in order to reduce social tension with the male members of the group.

The study group spent more time grooming on non-visible areas than visible areas. It clearly reflects the occurrence of allogrooming more than autogrooming, because all the grooming on the non-visible sites constitute allogrooming. Similarly, captive bonnet macaques also choses to groom more frequently in the head and neck (non-visible region) than the other visible regions (Dileep and Jose, 2014).

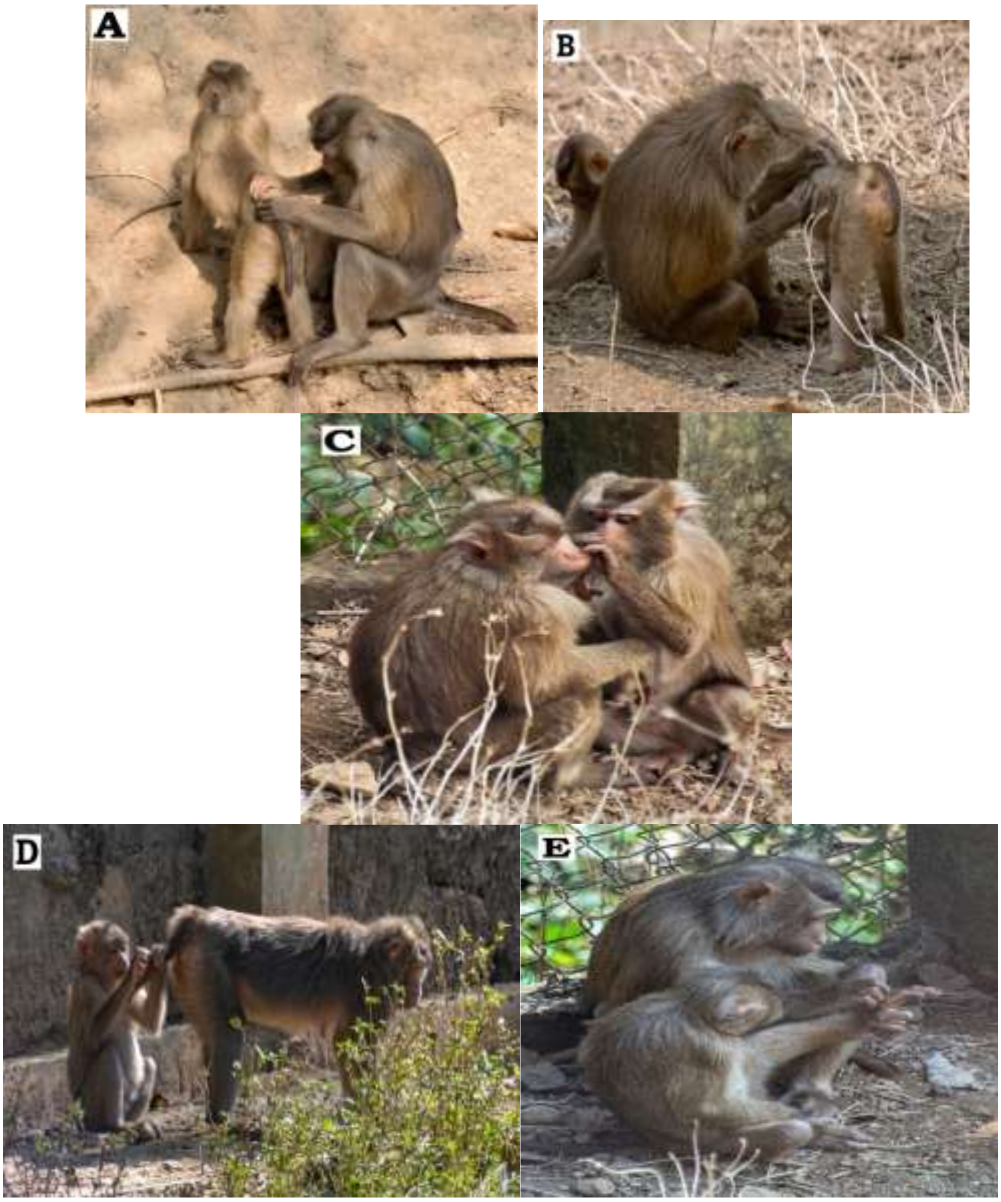


Photo plate 3: Northern pigtailed macaques performing allogrooming

A: Grooming non-visible region (Tail base)

B: Grooming non-visible region (Back with anal region)

C: Grooming non-visible region (Face)

D: Grooming non-visible region (Tail tip)

E: Grooming visible region (Feet)

CHAPTER VIII

GESTURAL COMMUNICATION

INTRODUCTION

Communication plays an important role in the social dynamics of primates. There are several modes of communication in animals. However, in primates, there are two major communication systems, namely, vocalization and gestures (Slocombe et al., 2011). Various authors working in the field of animal communication have suggested that language evolved from primate vocalizations (Aiello and Dunbar, 1993; Burling, 1993; Dunbar, 1996; Zuberbühler, 2005). Based on the intuitive link between vocalization and spoken language, research efforts have focused on vocal communication in different species of monkeys and apes (Kudo, 1987; Cheney and Seyfarth, 1988; Hammerschmidt and Fischer, 1998; Crockford and Boesch, 2003; Slocombe and Zuberbühler, 2005). However, the complex role that gestures play in communication among non-human primates has led a number of other theorists to propose that language evolved from the gestures rather than the vocal domain (Hewes, 1973; Kimura, 1993; Armstrong et al., 1995; Kendon, 2004; Corballis, 2002, 2010). The onset of communication behaviour in pre-linguistic children occurs through gestures much earlier than spoken words develop, suggesting that gestures are probably older link to language formation (Werner and Kapland, 1972; Bates et al., 1975; Camaioni, 1997). It has also been suggested that the symbolic nature of human language perhaps evolved from visual gestures in the evolutionary lineage of humans (Armstrong et al., 2007; Meir et al., 2013). The high rate of success in using gestures for training apes to learn human language suggests the gestural origins of human language to be of greater merit than that of primate vocalizations (Hayes, 1951; Gardner and Gardner, 1969; Patterson, 1978; Greenfield and Savage-Rumbaugh, 1990; Lyn et al., 2010). Hence, increasing attention is now being paid to the gesturing of non-human primates (Maestripieri, 1996; Tanner, 2004; Meguerditchian and Vauclair, 2006, 2009; Call and Tomasello, 2007; Pollick and de Waal, 2007; Laidre, 2008; Genty et al., 2009; Cartmill and Byrne, 2010; Meguerditchian et al., 2010). Similar to humans, their gesturing involves variable behavioural strategies. New gestures are invented, used for multiple purposes and adjusted to allow for the attentional state of the receiver (Tomasello and Zuberbühler, 2002; Call and Tomasello, 2007; Pollick and de Waal, 2007; Genty et al., 2009). Macaques use gestures to mediate both competitive and cooperative interactions with other group members. Gestures not only convey information on the emotional state of the sender and its impending behaviour, but can also be used to inhibit the behaviour of another individual or to request its participation in specific activities such as grooming, agonistic support, mating or play (Maestripieri, 1997).

Macaque lives in groups and they need to cooperate for protection from predators and /or defense of food resources from other con-specifics (Wrangham, 1987). Limited resources such as food, shelter and mates, however, inevitably lead group members to compete with each other. Communication is an adaptation to social life and mediates both cooperative and competitive interactions with con-specifics. Two basic functions of communication are to bring individuals together when there is need for cooperation and to keep them apart whenever competition arises. Many of the complexities of macaque social life and communication results from the elaboration of this simple system of approach and avoidance (Maestripieri, 1997). This study may provide insights for better understanding of the different types of gestures used for communication and their importance in the life of social animals such as primates.

MATERIALS AND METHODS

Subjects

Study on the gestural communications was done on 12 northern pigtailed macaques, which comprises of 3 adult males, 4 adult females, 3 juvenile males and 2 juvenile females. For this study, Focal sampling method (Altmann, 1974) was used for all activities associated with gestural signals.

Data collection

Study on the different gestural signals and the context of gestural signals was done for 15 months (May, 2016 to August, 2017). Observations were done on a 10-minute interval for 11 hours in a day (7:00 a.m. to 6:00 p.m.), and 6 days in a week, for all the gestural signals exhibited, the different contexts (occurrence) of the gestural signals, and modality (mode of exhibiting gestural signals, i.e., visual or tactile) of the gestures. Visual mode involves the gestures where body contact between the sender and receiver of gestural signals does not occur, and tactile mode involves the exhibition of gestural signals using body contact. The definitions of different gestural signals and the occurrences of the gestures are listed in Table 15.

Table 15: Types of gestures, actions during gestures and the context of communications

Gesture	Actions during communications	Context of communication
Lipsmack	Rapid opening and closing of the mouth and lips, such that when the lips close they make an audible smacking sound.	Aggression, Approach, after attacking
Pucker	The lips are compressed and protruded, the eyebrows, forehead and ears are retracted.	Grooming, playing, agonistic support
Teeth Chatter	The mouth is rapidly opened and closed and the lips are retracted, exposing the teeth	Submission
Bared teeth	The mouth is closed and the lips and lip corner are retracted so that the teeth are exposed in a white band.	Aggression, approach,
Eyebrows	The scalp and brows are retracted and the mouth is open.	Agonistic support
Touch face	One hand is extended to touch the face of another individual while standing and sitting in front of it.	Play, Sexual communication
Touch genitals	Manipulation of the genitals of another individual without olfactory inspection.	Sexual communication
Present	The tail is raised to expose the genitals.	Submission, sexual communication
Mock bite	Gripping another individual's skin with the teeth, slowly, without roughness, for several seconds.	Playing, after attacking
Embrace	Ventral embrace with both arms around the torso of another individual, in the sitting position and kneading the partner's fur or flesh.	Support, grooming
Mount	Mount with or without foot clasp but with no intromission of thrusts.	Dominance, sexual communication

Data analysis

To determine the difference in the amount of time each gestural signal was exhibited among the different age groups, Kruskal-Wallis test was employed. The significant differing levels of gestural signals as indicated by Kruskal-Wallis test were subjected to pairwise comparison by employing Mann-Whitney test. All the statistical analyses were performed on SPSS ver. 18.0.

RESULTS

Types of gestural signals

Eleven types of gestural signals were observed during the study period. The number of times each gestural signal was exhibited by different age groups is given on table 16. The percentage of time that each gestural signal is exhibited by different age groups is given on table 17. Lipsmack, bared-teeth and pucker were the gestural signals that were observed most frequently in the study group. Adult males showed lipsmack (32.13%) the most among all the types of gestures that was observed. Adult females (29.74%), juvenile males (26.81%) and juvenile females (27.09%) exhibited pucker the most among all the gestures. Kruskal-Wallis test was carried out for the different gestures observed (Table 18). Kruskal-Wallis test revealed significant variation on the amount of teeth chatter, present and mount observed in the age groups ($p<0.05$), which were subjected to Mann-Whitney pairwise test (Table 19). Teeth chatter and present gestures were observed in adult males significantly less frequent ($p<0.05$) than the other age groups. Juvenile males showed significantly less present than adult females and juvenile females ($p<0.05$).

Mount gesture was observed significantly more frequent in adult males than all the age groups ($p<0.05$), juvenile males showed more mount than both the adult females and juvenile females ($p<0.05$).

Table 16: Amount of time each gestural signals was exhibited by different age groups

Type of gestures	Number of gestural signals exhibited			
	Adult males	Adult females	Juvenile males	Juvenile females
Lipsmack	1033	554	756	435
Pucker	485	987	879	891
Teeth chatter	32	182	281	335
Bared teeth	818	395	466	267
Eyebrows	100	241	165	242
Present	7	468	19	606
Mock bite	103	142	197	211
Embrace	107	221	107	174
Mount	409	0	257	0
Touch face	23	116	59	105
Touch genitals	98	13	93	23
Total	3215	3319	3279	3289

Table 17: The amount of time each gestural signal was exhibited by different age groups (percentage)

Type of gestures	Amount of time (percentage)			
	Adult males	Adult females	Juvenile males	Juvenile females
Lipsmack	32.13	16.69	23.06	13.23
Pucker	15.09	29.74	26.81	27.09
Teeth chatter	1.00	5.48	8.57	10.19
Bared teeth	25.44	11.90	14.21	8.12
Eyebrows	3.11	7.26	5.03	7.36
Present	0.22	14.10	0.58	18.43
Mock bite	3.20	4.28	6.01	6.42
Embrace	3.33	6.66	3.26	5.29
Mount	12.72	0.00	7.84	0.00
Touch face	0.72	3.50	1.80	3.19
Touch genitals	3.05	0.39	2.84	0.70

Table 18: Kruskal-Wallis test of the different gestural signals by different age groups

Gestural signals	$\chi 2$	df	p value
Lipsmack	0.128	3	0.988
Pucker	2.21	3	0.532
Teeth chatter	8.427	3	0.038*
Bared teeth	0.785	3	0.853
Eyebrows	1.564	3	0.668
Present	9.392	3	0.025*
Mock bite	0.607	3	0.895
Embrace	5.051	3	0.169
Mount	10.649	3	0.014*
Touch face	2.414	3	0.491
Touch genitals	7.416	3	0.06

$\chi 2$= Chi square value, p= Probabiliy value

Table 19: Mann-Whitney pairwise test for teeth chatter, present and mount observed in different age groups

Age groups	Teeth chatter			Present			Mount		
	U	W	p	U	W	p	U	W	p
AM vs AF	0.000	6.000	0.050*	0.000	6.000	0.046*	0.000	6.000	0.037*
AM vs JM	0.000	6.000	0.049*	0.000	6.000	0.046*	0.000	6.000	0.050*
AM vs JF	0.000	6.000	0.049*	0.000	6.000	0.046*	0.000	6.000	0.037*
AF vs JM	0.500	6.500	0.077	0.000	6.000	0.050*	0.000	6.000	0.037*
AF vs JF	2.000	8.000	0.275	4.000	10.000	0.827	4.500	10.500	1.000
JM vs JF	2.000	8.000	0.275	0.000	6.000	0.050*	0.000	6.000	0.037*

U=Mann-Whitney U, W=Wilcoxon W, p= Probability value
AM-Adult males, AF- Adult females,
JM- Juvenile males, JF- Juvenile females

Context/Occurrence of gestures

9 types of context of gestures were recorded, and the frequency of these incidences for each age groups were also recorded (Table 20). Aggression was the most frequent context of gestural signals observed in adult males (29.16%) and juvenile males (24.04%), followed by approach (adult males: 21.27%; juvenile males: 21.01%). Gestural signals in the context of grooming was the most frequent context observed in both adult females (21.77%) and juvenile females (20.57), followed by aggression in adult females (21.08%) and submission in juvenile females (20.19%). Kruskal-Wallis test was carried out for the different contexts of the gestural signals and is given on table 21. Kruskal-Wallis test revealed significant

variation on the amount of playing, approach and dominance by the different age groups. These variations were subjected to Mann-Whitney pairwise test (Table 22). Playing was observed significantly less in adults of both sexes than the juvenile males and juvenile females ($p<0.05$). The occurrence of gestures as a result of approach was significantly more in adult males than juvenile males and juvenile females ($p<0.05$), and it is lower in juvenile females as compared to adult females and juvenile males ($p<0.05$). The occurrences of gestures in the context of dominance was significantly higher in adult males as compared to the other age groups ($p<0.05$), and higher in adult females compared to both juvenile males and juvenile females ($p<0.05$).

Table 20: Frequency of different contexts of gestural signals within the different age groups

Occurrence of incidence/Context	**Frequency of contexts (%)**			
	Adult males	**Adult females**	**Juvenile males**	**Juvenile females**
Playing	6.05	8.18	13.84	12.41
Aggression	29.16	21.08	24.04	19.14
Approach	21.27	13.41	21.01	9.71
Grooming	9.08	21.77	9.74	20.57
Submission	2.51	19.16	7.61	20.19
Agonistic support	10.75	6.62	9.43	8.25
After attacking	2.62	2.78	2.43	2.36
Dominance	12.3	0.17	8.21	0.52
Sexual communication	6.21	6.79	3.65	6.81

Table 21: Kruskal-Wallis test for different occurrences of gestural signals

Occurrence of incidence	$\chi 2$	df	p value
Playing	8.916	3	0.030*
Aggression	5.974	3	0.113
Approach	8.967	3	0.030*
Grooming	6.137	3	0.105
Submission	6.034	3	0.110
Agonistic support	5.378	3	0.146
After attacking	2.613	3	0.455
Dominance	9.755	3	0.021*
Sexual communication	0.913	3	0.822

$\chi 2$= Chi square value, p= Probabiliy value

Table 22: Mann-Whitney pairwise comparison of different contexts of gestural signals

AGE GROUPS	PLAYING			APPROACH			DOMINANCE		
	U	W	p	U	W	p	U	W	p
AM vs AF	3.000	9.000	0.513	2.000	8.000	0.275	0.000	6.000	0.043*
AM vs JM	0.000	6.000	0.049*	0.000	6.000	0.050*	0.000	6.000	0.046*
AM vs JF	0.000	6.000	0.050*	0.000	6.000	0.049*	0.000	6.000	0.043*
AF vs JM	0.000	6.000	0.049*	1.500	7.500	0.184	0.000	6.000	0.046*
AF vs JF	0.000	6.000	0.050*	0.000	6.000	0.050*	4.500	10.500	1.000
JM vs JF	1.500	7.500	0.184	0.000	6.000	0.050*	0.000	6.000	0.046*

U=Mann-Whitney U, W=Wilcoxon W, p= Probability value

AM=Adult males, AF= Adult females, JM= Juvenile males, JF= Juvenile females

Modality of gestures

The study group uses two modes of exhibiting gestural signals, i.e., visual and tactile. Visual mode of gestures was observed more frequently than tactile mode of gestures in adult males (figure 4). The pairwise test for each age group is given on table 23.

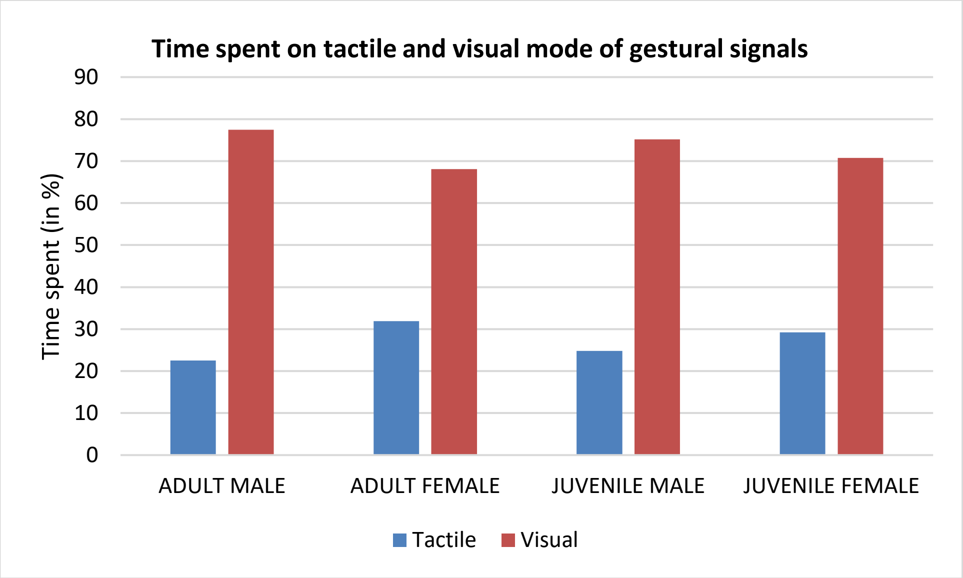


Figure 4: Time spent on the two modes of gestures in all the age groups

Table 23: Mann-Whitney test for tactile and visual mode of gestural signals in different age groups

Age groups	U	W	p
Adult males	0.000	6.000	0.050*
Adult females	0.000	6.000	0.050*
Juvenile males	0.000	6.000	0.050*
Juvenile females	0.000	6.000	0.046*

U=Mann-Whitney U, W=Wilcoxon W, p= Probability value

DISCUSSIONS

It is important to have better understanding of the different types of gestures used for communication and their importance in northern pigtailed macaque to help in the maintenance of the animals in captivity. Knowledge on the gestural communication may also help researchers in the field of human language evolution. In macaques, competition occurs not only between individuals but also between families and groups. Therefore, gestures may be used not only to communicate with competitors but also with relatives, friends and allies who may provide their support in agonistic encounters (Maestripieri, 1997). 11 types of gestural signals were observed in the study. Variations in the types of gestural communications is dependent on environment conditions and other biotic parameters. Findings of the present study and previous researches on macaques has shown the existence of variation in the frequency and types of different gestural signals, and even within the same species of different regions. Goosen and Kortmulder (1979) recorded 19 different facial expressions in pigtailed macaque. Maestripieri (1996) and Maestripieri (2005) reported 13 types of gestures and 8 types of gestures in pigtailed macaques, 12 types of gestures in stumptailed macaques and 4 types of gestures in rhesus macaques. Lalremruati et al. (2017) reported 9 types of gestural signals in assamese macaques and 8 types of gestural signals in rhesus macaques. 24 gestural signals were exhibited by bonnet macaques in the wild (Gupta, 2015). Captive groups of animals have limitations in socialization, aggressions and other activities, hence, the types of gestural signals observed is less in the present study than the other studies conducted in the wild monkeys.

Lipsmack and pucker were the gestural signals that were observed most frequently in the study group, which is the case with Maestripieri (1996), which reported lipsmack, pucker and bared-teeth to be the most frequent gestural signals in pigtailed macaque. In the present study, adult males exhibited lipsmack (32.14%) most frequently among all the types of gestures that they exhibited. Lipsmack occurred in the context of aggression and approach after aggression in most of the instances, therefore, it can be an indicator of the need for adult males to stay aggressive to keep other members of the group in control, and to maintain strict hierarchy in the group. Accordingly, lipsmack was one of the most frequent gestural signals in rhesus macaques (Maestripieri, 2005; Lalremruati et al., 2017), assamese macaques (Maestripieri, 2005; Lalremruati et al., 2017), stumptailed macaques (Maestripieri, 2005) and bonnet macaques (Gupta, 2015). Adult females (30.08%), juvenile males (26.81%) and juvenile females (27.36%) exhibited pucker the most among all the gestures. Pucker gesture is observed in the social contexts such as grooming, support and playing. This result may reflect the role of adult females in keeping the group socially close together, and that the females and the subordinate males are more social and are more involved in interactions with other group members than the adult males. Pucker has been reported as one of the most frequent gesture observed in pigtailed macaque (van Hooff, 1962; Bobbitt et al., 1964; van Hooff, 1967; Kaufman and Rosenblum, 1966; Bernstein, 1967; Maestripieri, 1996; 2005). While pucker is a common gesture in pigtailed macaque and liontailed macaques (Johnson, 1985; Lindburg et al., 1985; Maestripieri, 1996; 2005; Lalremruati et al., 2017), it is rare in rhesus macaques and longtailed macaques (Shirek-Ellefson, 1972; Maestripieri, 2005, Lalremruati et al., 2017), and has not been reported in stumptailed macaque (Maestripieri, 2005), assamese macaque (Lalremruati et al., 2017) and bonnet macaques (Gupta, 2015). This may suggest that pucker maybe a relatively ancestral signal that has been conserved in the *Silenus* group of macaques but partially lost and non-existent in other macaque species.

Teeth chatter gesture has not been reported in pigtailed macaque previously, even though it has been reported in other macaque species such as stumptailed macaque, barbary macaques, bonnet macaques, Tibetan macaques and assamese macaques (van Hoof, 1967; Fooden, 1980; Maestripieri, 2005). Maestripieri (2005) suggested that since the teeth-chatter gesture has been reported previously only in the mentioned macaques which are believed to be the most ancestral macaque species and in macaque species of the sinica group; teeth-chatter evolved relatively early in macaques, was retained in Barbary macaques and species of the sinica group, and was lost in other species such as rhesus macaques and pigtailed macaques. However, the present study reports the occurrence of this gesture in pigtailed macaques. Van Hoof (1967) further indicated that different macaque species may have independently evolved teeth-chatter from other gestural signals such as bared-teeth and lipsmack.

Mann-Whitney test revealed significantly more gestural signals in the context of playing by juvenile males and juvenile females than both the adult males and females. Juveniles tend to be generally energetic, active and playful than the adults. They spent a large proportion of their total time budget for play associated behaviours (Worlein and Sackett, 1997). The occurrence of gestural signals in the context of approach and dominance was significantly higher in adult males than the other age groups. This finding further indicates the presence of well-defined hierarchy in the group. The use of visual mode of gesture was significantly more frequent than the tactile mode of gestures in all the age groups, which may indicate the importance of facial expressions in macaques and primates in general. Primates have been reported to produce facial expressions in greater abundance and variety than other animal groups (Darwin, 1872; van Hoof, 1967; Burrows, 2008).

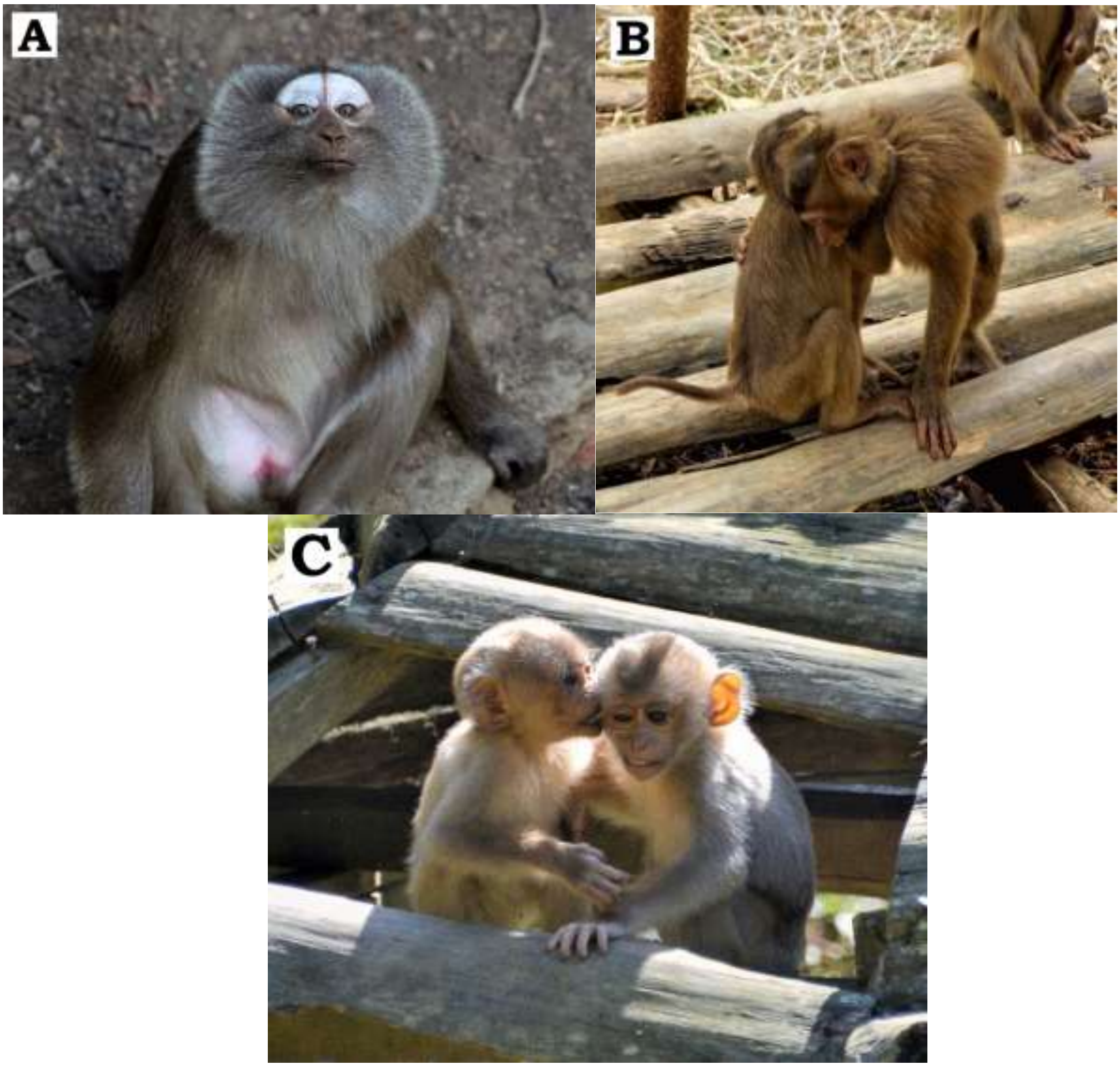


Photo plate 4: Gestural signals in northern pigtailed macaques

A: Pucker

B: Mockbite in two juvenile males

C: Mockbite in two infants

CHAPTER IX

MOTHER-INFANT RELATIONSHIP

INTRODUCTION

Female parental care is the rule among most of the nonhuman primates, exceptions being a few socially monogamous species in which fathers care for their infants. Mother-infant relationships have a lasting and versatile impact on the survival of the infant in both physical and psychological ways. Therefore, it is very important to understand what influences the relationship and how it influences the behaviour and development of offspring. Primate infants are born dependent on their mother (Strier, 2007) and mothers play a role throughout their offspring's lives (Hrdy, 1999). Maternal behaviour is critical for infant survival largely in mammals (Nicolson, 1991). In most species, females perform the greatest share of infant caretaking. With the evolution of lactation, the behaviour and physiology of mammalian mothers and their young are inextricably linked together in unique adaptive complex (Pond, 1977). The milk of primates is high in carbohydrates but low in fat and protein. Among the Old-World monkeys and the apes, females typically give birth to one infant at a time, feed their offspring with milk and provide physical support and continue investing in them till the infants become independent with little or no contribution from males. In contrast to some mammalian species whose young are cached in nests or dens, primate neonates are in constant contact with their mothers. Behavioural adaptations such as the clinging/grasp reflex and infant vocalizations serve to maintain contact. Mothers play an important role in the thermoregulation of the infants by the extensive body contact between them. In the wild, primate mothers carry infants during long journeys, over difficult passages and away from predators even after infants are old enough to locomote skillfully on their own (Nicolson, 1991). Primate mothers play an important role in the development and independence by socializing their infants through modelling appropriate social behaviours and feeding techniques, sharing feeding sites or food items, and actively encouraging independence. Although general course of development of mother-infant relationship is extremely steady across the species (Altmann, 1980; Dunbar, 1988), individual mother-infant relationship varies substantially within groups. Inter-individual differences in mother-infant interactions among non-human primates can often be described in terms of particular qualities of relationship that is generally based on the identification of differences in one or more measures of mother-infant interactions between individual mother-infant pairs. The measures are concerned with the regulation of contact and proximity between mothers and infants (Hinde and Simpson, 1975).

Allomothering is a kind of carrying infants by females other than the mother, where the allomothers care for the infants and also support the biological mother (Hrdy, 1999; Bentley-Condit et al., 2001). Small (1990) discussed the relationship between non-mother and infant widely in genus *Macaca* and recorded permissive mothering with infant transfer in some species while in others, the infant rarely comes in contact with other members of the group. Females groom others' infants (natal attraction), which can lead to holding and carrying of these infants in a manner that resembles maternal care (infant handling) (Hrdy, 2009). While natal attraction shows an individual's interest in an infant, infant handling also depends on whether the mother is willing to allow independent interactions between her infant and the handler (Maestripieri, 1994; Hrdy, 2009). Hence, the females who show the most natal attraction are not necessarily the ones who most often get to handle infants (Maestripieri, 1994). Infants' age and sex may also influence natal attraction and infant handling. Allomothering care can be biased toward male or female infants when investing in one sex over the other later leads to greater fitness outcomes for the caregiver (Bercovitch, 2002). The aim of this study is to understand the relationships of mother and infants and their impacts on the development and survival of infants; and the interaction of other females (non-mothers) with the infants in captive northern pigtailed macaques. The knowledge on these aspects and contributing factors in mother-infant relationships may help better planning of management for captive populations.

MATERIALS AND METHODS

Subjects

The study was done on five mother-infant pairs. During the whole study period, five infants were born, which are described below. All the births occurred during March to September.

- Infant 1- male (mother- Nutei)
- Infant 2- female (mother-Luna)
- Infant 3- male (mother- Ginny)
- Infant 4- female (mother- Nutei)
- Infant 5- male (mother-Luna)

Data collection

Observations were recorded by focal sampling method (Altmann, 1974) for eleven measures of mother-infant interactions- Total time in contact, total time off contact, on nipple contact, off nipple contact, off contact to touching distance (less than 3 feet away), off contact beyond touching distance (more than 3 feet away), total contact broken, leaves by mother, leaves by infant, approach by mother and approach by infant, for the first 6 months of infants life. Observations were also recorded by sampling all occurrence method (Altmann, 1974) for social interactions between the non-mother females and the infants (allomothering), such as grooming, touch-hand, sitting-touching, embrace and genital stimulation.

All the observations were carried out for 11 hours a day (7:00 a.m. to 6:00 p.m.) every day for the first 6 months of the infants' life.

Data analysis

Spearman correlation test was performed to examine the correlation between the infants' age and the mother-infant interactions, and also the frequency of care contacts received from the non-mother females. Mann-Whitney test was employed to determine the pairwise variations in mother-infant relationships between infants born to the same mother. Mann-Whitney test was performed to determine the influence of infants' sex on the amount of care contact received from non-mother females. All the statistical analyses were done using SPSS ver.18.

RESULTS

Infants' age and mother-infant relationships

The different measures of mother-infant relationships, such as time spent in mother's contact, on nipple contact, off nipple contact, time spent off mother's contact, time spent on touching distance, time spent beyond touching distance, total number of contact broken, contact broken by mother, contact broken by infant, approach by mother and approach by infant with age of the infants (months) for the 5 infants observed are given on tables 24 to 34. The pattern of variation among the 5 mother-infant pairs for all the mother-infant interactions are given on figures 5-15. The behaviour and development of the infants during the study period were more or less similar in the different aspects with increasing age.

Table 24: The total time spent on mother's contact by infants

Name of infant	Time (percentage)					
	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Infant 1	94.36	82.80	68.80	46.58	31.01	18.38
Infant 2	98.17	87.74	74.29	62.93	46.06	36.98
Infant 3	97.45	85.36	73.24	61.36	39.26	22.17
Infant 4	98.19	84.36	71.29	58.16	43.75	34.10
Infant 5	92.82	75.81	68.45	50.15	37.42	15.73

Table 25: The time spent on nipples contact by infants

Name of infant	Time (percentage)					
	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Infant 1	91.98	78.57	61.45	45.86	35.96	19.09
Infant 2	90.78	81.25	69.84	52.80	47.02	36.13
Infant 3	81.56	64.70	56.39	46.64	35.94	17.26
Infant 4	93.52	83.60	75.51	66.13	39.36	22.22
Infant 5	80.87	69.49	60.37	52.70	34.74	20.00

Table 26: The time spent off nipples contact by infants

Name of infant	Time (percentage)					
	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Infant 1	8.02	21.43	38.55	54.14	64.04	80.91
Infant 2	9.22	18.75	30.16	47.20	52.98	63.87
Infant 3	18.43	35.29	43.61	53.36	64.06	82.74
Infant 4	6.48	16.40	24.49	33.87	60.64	77.78
Infant 5	19.13	30.51	39.63	47.30	65.26	80.00

Table 27: The total time spent off mother's contact by infants

Name of infant	Time (percentage)					
	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Infant 1	5.64	17.2	31.2	53.42	68.99	81.62
Infant 2	1.83	12.26	25.71	37.07	53.94	63.02
Infant 3	2.55	14.64	26.76	38.64	60.74	77.83
Infant 4	1.81	15.64	28.71	41.84	56.25	65.9
Infant 5	7.18	24.19	31.55	49.85	62.58	84.27

Table 28: The time spent on touching distance by infants

Name of infant	Time (percentage)					
	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Infant 1	63.16	52.94	50.57	33.05	18.06	14.69
Infant 2	56.00	37.80	36.79	25.75	16.49	9.33
Infant 3	69.23	55.32	52.50	42.98	19.05	10.30
Infant 4	66.67	50.00	52.56	46.15	34.86	26.13
Infant 5	75.00	66.67	38.46	35.66	31.46	13.39

Table 29: The time spent beyond touching distance by infants

Name of infant	Time (percentage)					
	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Infant 1	36.84	47.06	49.43	66.95	81.94	85.31
Infant 2	44.00	62.20	63.21	74.25	83.51	90.67
Infant 3	30.77	44.68	47.50	57.02	80.95	89.70
Infant 4	33.33	50.00	47.44	53.85	65.14	73.87
Infant 5	25.00	33.33	61.54	64.34	68.54	86.61

Table 30: Total contact broken by mother-infant pairs

Name of infant	Contact broken (in numbers)					
	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Infant 1	13	23	30	36	45	53
Infant 2	4	10	20	29	35	44
Infant 3	18	25	33	33	43	58
Infant 4	11	19	25	32	39	51
Infant 5	15	24	31	39	43	55

Table 31: Total contact broken by mother

Name of infant	Contact broken (in numbers)					
	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Infant 1	2	7	9	11	14	13
Infant 2	0	3	6	8	11	15
Infant 3	4	6	7	9	11	17
Infant 4	3	9	10	13	15	16
Infant 5	5	7	9	14	11	12

Table 32: Total contact broken by infant

Name of infant	Contact broken (in numbers)					
	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Infant 1	11	16	21	25	31	40
Infant 2	4	7	14	21	24	29
Infant 3	14	19	26	24	32	41
Infant 4	8	10	15	19	24	35
Infant 5	10	17	22	25	32	43

Table 33: Total number of approach by mother

Name of infant	Time (percentage)					
	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Infant 1	8	15	19	21	20	22
Infant 2	2	6	13	17	14	20
Infant 3	4	10	15	17	19	25
Infant 4	6	7	9	13	14	17
Infant 5	9	9	13	15	14	21

Table 34: Total number of approach by infant

Name of infant	Time (percentage)					
	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Infant 1	5	8	11	15	25	31
Infant 2	2	4	7	12	21	24
Infant 3	14	15	18	20	24	33
Infant 4	5	12	16	19	25	34
Infant 5	6	15	18	24	29	34

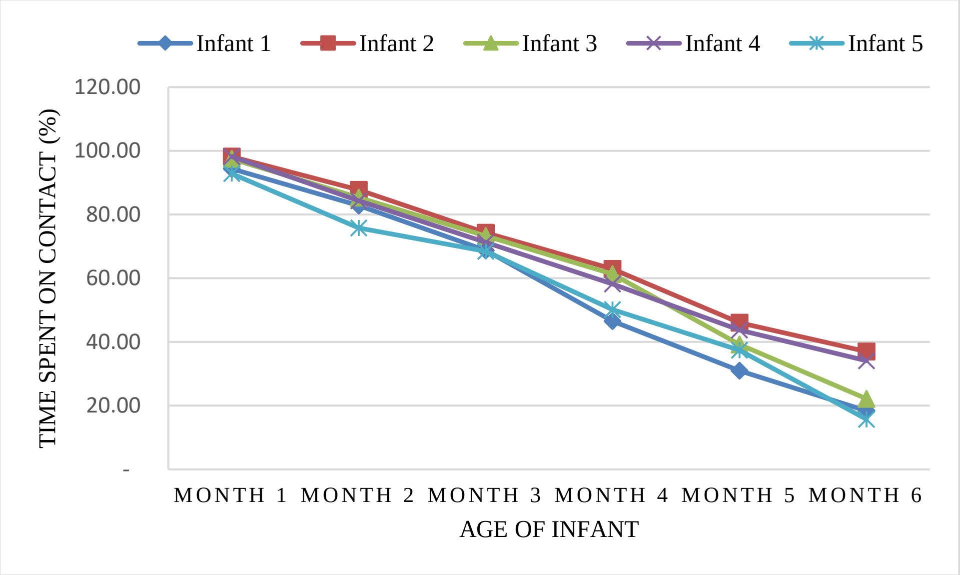


Figure 5: Month wise time spent in mother's contact

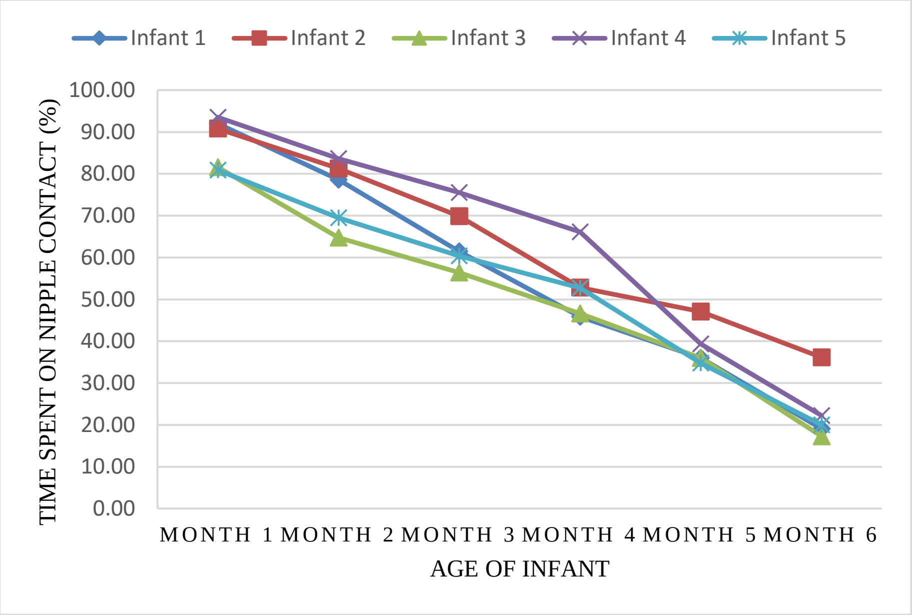


Figure 6: Month wise time spent on nipple contact

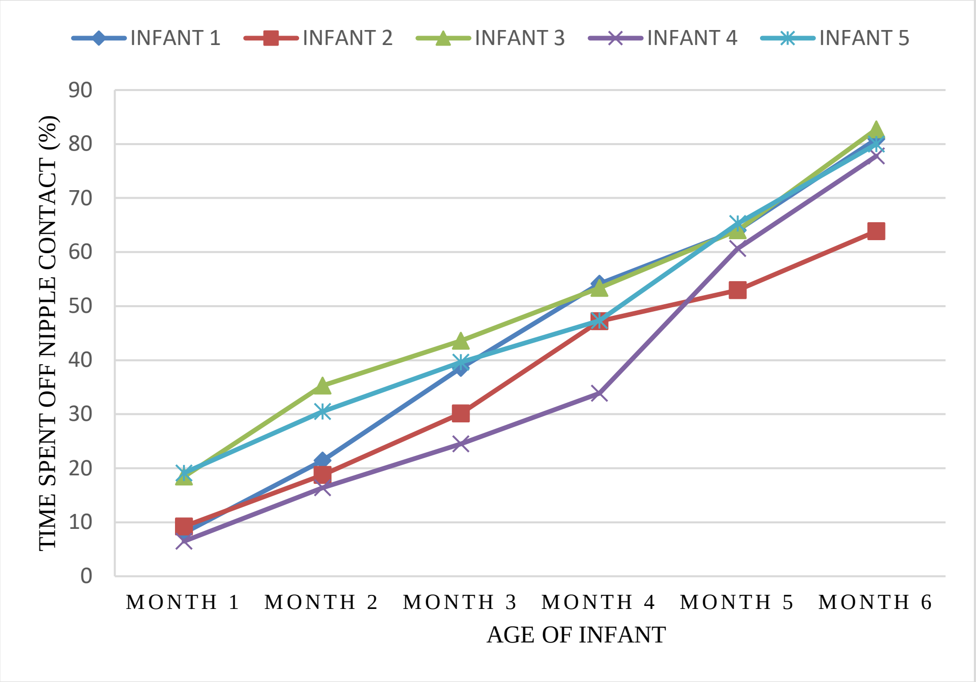


Figure 7: Month wise time spent off nipple contact

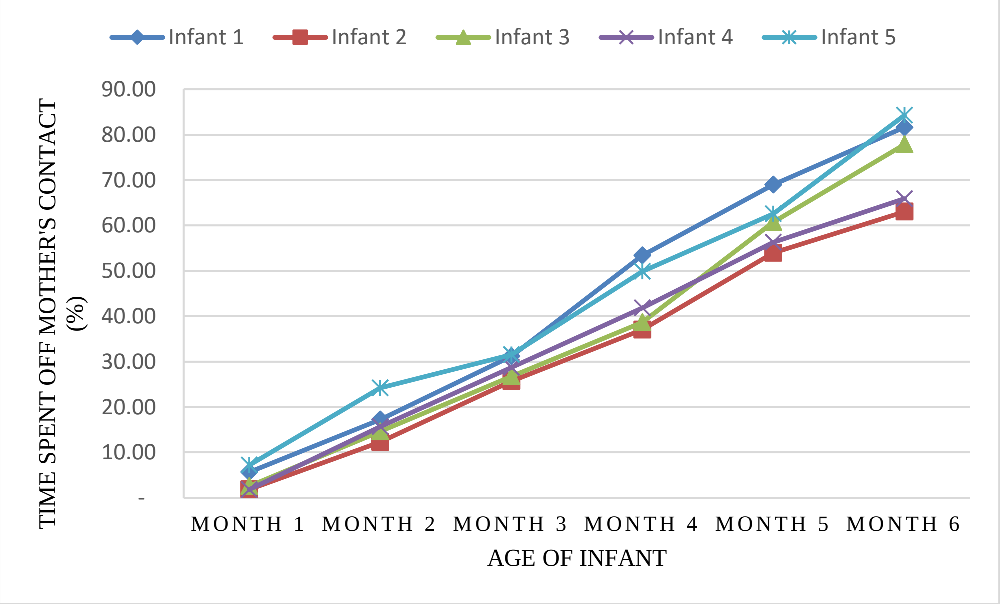


Figure 8: Month wise time spent off mother's contact

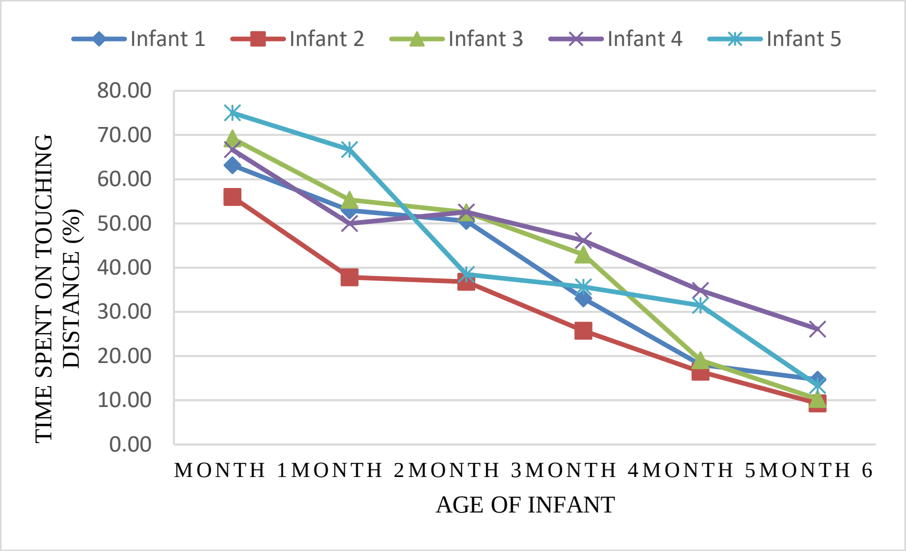


Figure 9: Month wise time spent on touching distance

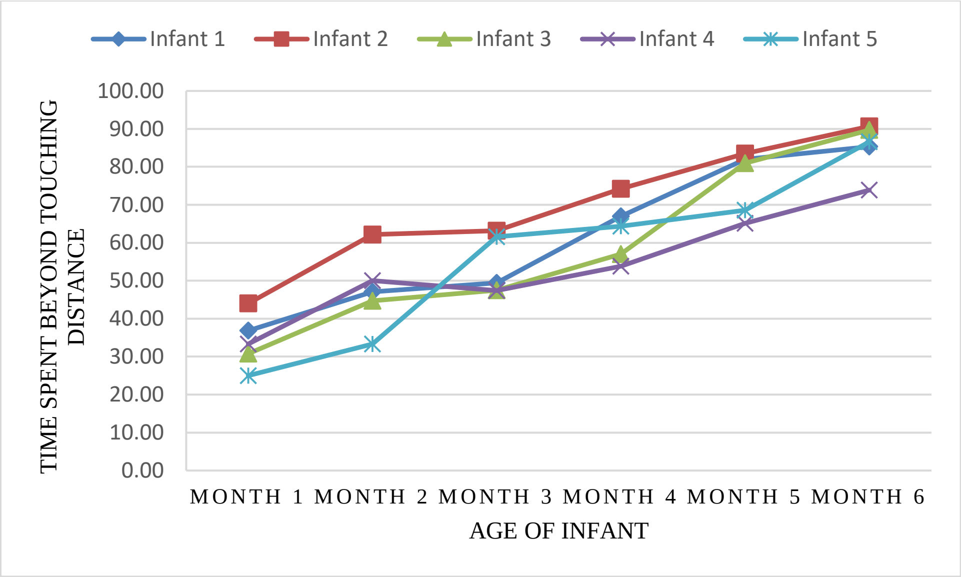


Figure 10: Month wise time spent beyond touching distance

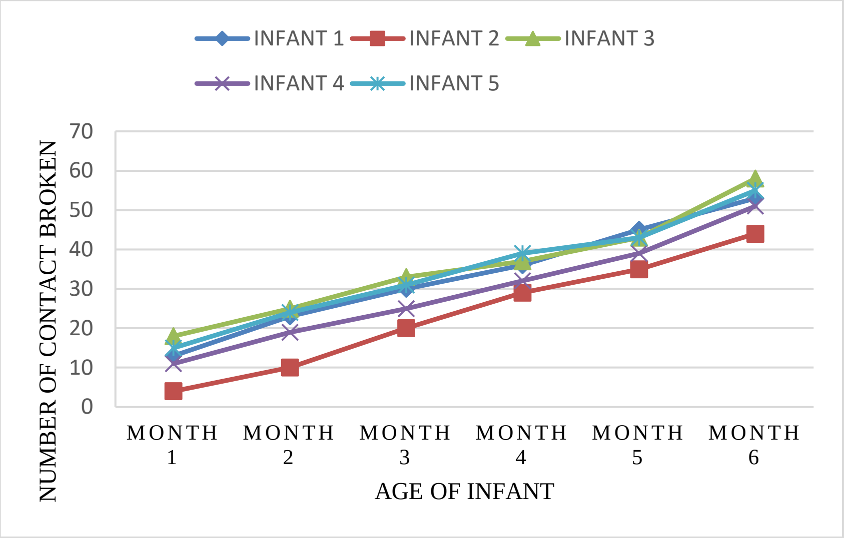


Figure 11: Month wise contact broken between the mother and infant

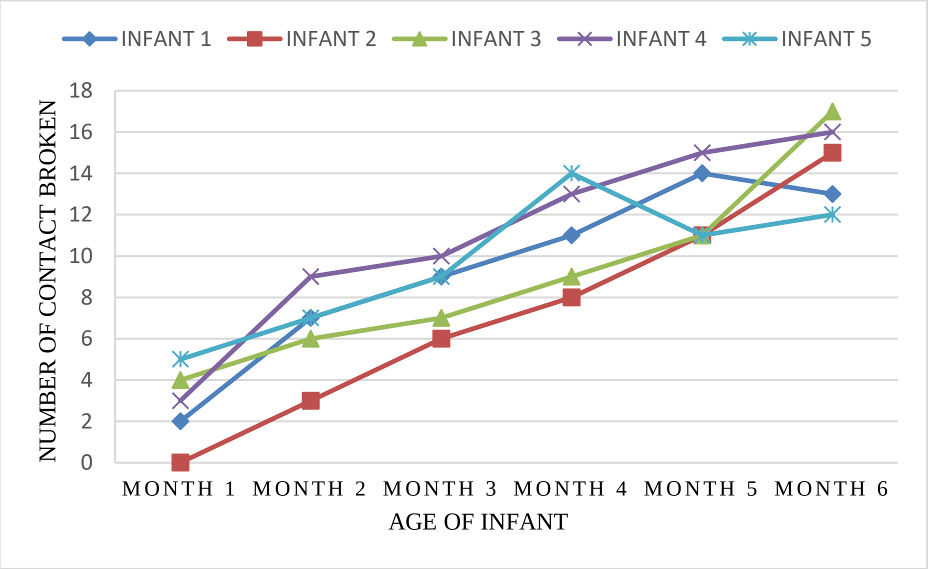


Figure 12: Month wise contact broken by mothers

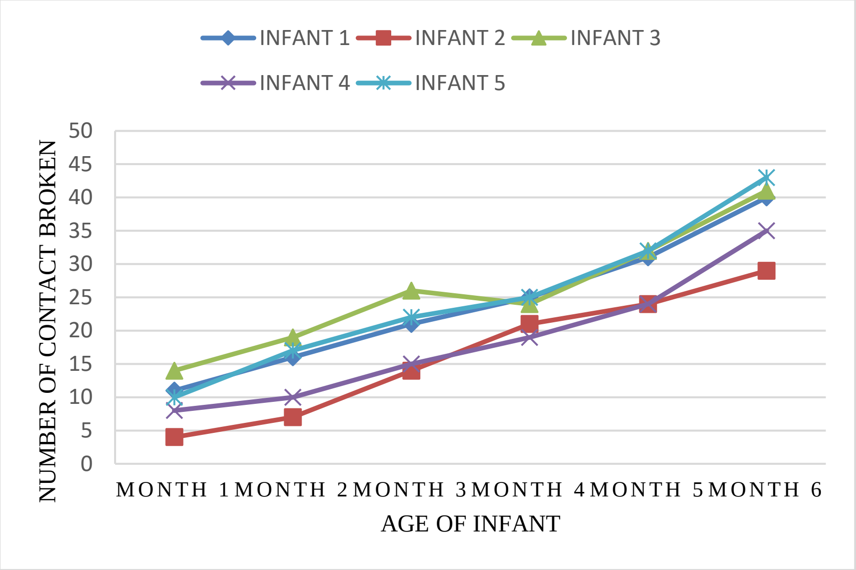


Figure 13: Month wise time contact broken by infants

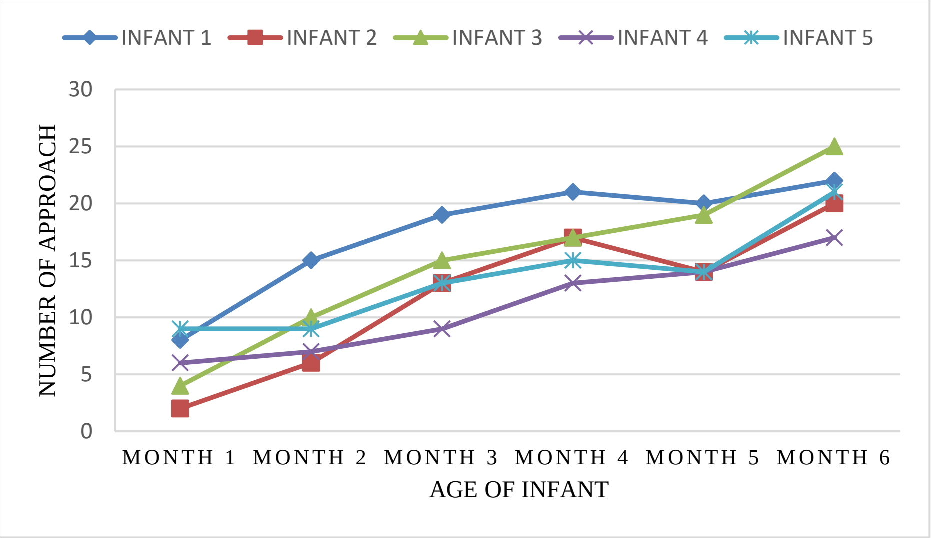


Figure 14: Month wise approach by mothers

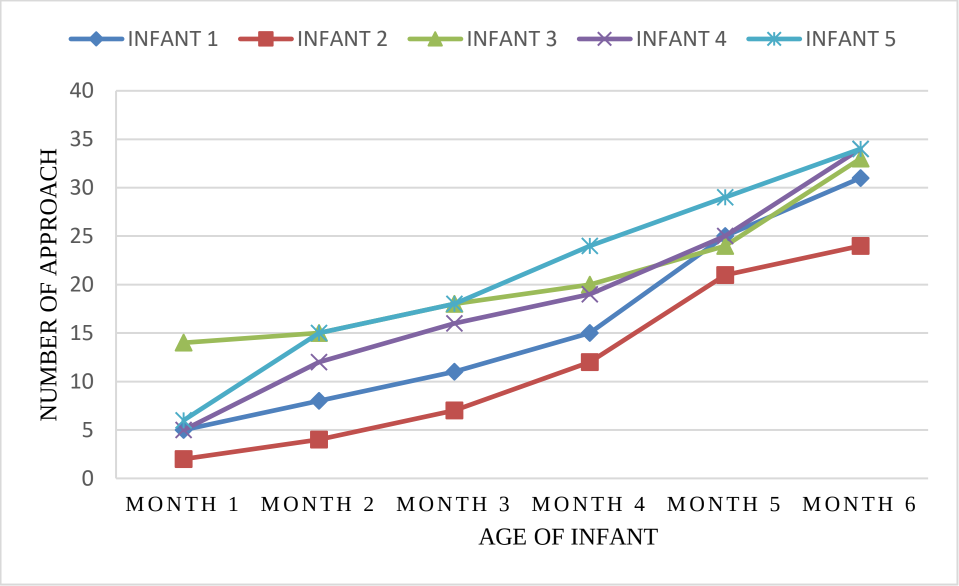


Figure 15: Month wise approach by infants

Spearman correlation tests between infants' age and the different measures of mother-infant relationship for all the 5 pairs is given on table 35. Spearman correlation tests revealed that the total time spent on contact and on nipple contact decreased significantly with increase in infants' age ($p<0.05$). The total time off contact, time spent beyond touching distance, total contact broken, leaves by infant and leaves by mother increases significantly with increase in infants' age ($p<0.05$). Individual variation exists on the time spent off nipple contact, number of approach by mother and number of approach by infants. Off nipple contact was found to decrease significantly ($p<0.05$) with increase in infants' age for infant 3, infant 4 and infant 5. Unusually, approach by mother significantly increases ($p<0.05$) with increase in infants' age for infant 4. Approach by infant also significantly increases ($p<0.05$) with increase in infants' age for infant 4 and infant 5. Kruskal-Wallis test was carried out for variations in all the mother-infant interactions among the different mother-infant pairs, which is given on table 36. However, there is no significant variation on the pattern of mother-infant relationships among the different mother-infant pairs.

Table 35: Spearman correlation between infants' age (month) and mother-infant relationship

Mother-infant interaction		rs	p value
Total time in contact	Infant 1	-1.000	0.001*
	Infant 2	-1.000	0.001*
	Infant 3	-1.000	0.001*
	Infant 4	-1.000	0.001*
	Infant 5	-1.000	0.001*
Total time off contact	Infant 1	0.943	0.005*
	Infant 2	1.000	0.001*
	Infant 3	1.000	0.001*
	Infant 4	0.829	0.042*
	Infant 5	1.000	0.001*
On nipple contact	Infant 1	-1.000	0.001*
	Infant 2	-1.000	0.001*
	Infant 3	-1.000	0.001*
	Infant 4	-1.000	0.001*
	Infant 5	-1.000	0.001*
Off nipple contact	Infant 1	-0.657	0.156
	Infant 2	-0.771	0.072
	Infant 3	-0.943	0.005*
	Infant 4	-1	0.001*
	Infant 5	-0.829	0.042*
On touching distance	Infant 1	0.029	0.957
	Infant 2	0.257	0.623
	Infant 3	0.257	0.623
	Infant 4	0.486	0.329
	Infant 5	0.657	0.156
Beyond touching distance	Infant 1	1	0.001*
	Infant 2	1	0.001*
	Infant 3	1	0.001*
	Infant 4	1	0.001*
	Infant 5	1	0.001*
Total contact broken	Infant 1	1	0.001*
	Infant 2	1	0.001*
	Infant 3	1	0.001*
	Infant 4	1	0.001*
	Infant 5	1	0.001*

Leaves by mother	Infant 1	0.943	0.005*
	Infant 2	1	0.001*
	Infant 3	1	0.001*
	Infant 4	0.829	0.042*
	Infant 5	0.943	0.005*
Leaves by infant	Infant 1	1	0.001*
	Infant 2	1	0.001*
	Infant 3	1	0.001*
	Infant 4	0.971	0.001*
	Infant 5	1	0.000*
Approach by mother	Infant 1	0.486	0.326
	Infant 2	0.6	0.208
	Infant 3	0.029	0.957
	Infant 4	0.829	0.042*
	Infant 5	0.771	0.072
Approach by infant	Infant 1	0.486	0.326
	Infant 2	0.543	0.266
	Infant 3	0.6	0.208
	Infant 4	0.943	0.005*
	Infant 5	0.899	0.015*

r_s= Spearman correlation value, p= Probability value

Table 36: Kruskal-Wallis test for the different mother-infant relationships among the different mother-infant pairs

Mother-infant interactions	χ^2 value	p value
Total time in contact	0.813	0.937
On nipple contact	2.095	0.718
Off nipple contact	2.095	0.718
Total time off contact	0.813	0.937
Touching distance	2.012	0.734
Beyond touching distance	2.012	0.734
Total contact broken	2.213	0.697
Contact broken by mother	2.239	0.692
Contact broken by infant	4.014	0.404
Approach by mother	5.453	0.244
Approach by infant	3.965	0.411

Influence of infants' sex on mother-infant relationships

Mann-Whitney pairwise test was performed for male infants and female infants born to the same mother to analyze the influence of infant's sex on the different parameters of mother-infant relationship (Table 37). During the study period, adult female 1 (Nutei) gave birth to a male infant (infant 1) and a female infant (infant 4). Adult female 2 (Luna) also gave birth to a female infant (infant 2) and a male infant (infant 5). Infant 1 was active and independent earlier than infant 4 even though they were born from the same mother. The number of time infant leaves (broken contact) was significantly more ($p<0.05$) in infant 1 than infant 4, the amount of time infant approach the mother was also significantly higher ($p<0.05$) in infant 4 than infant 1. The time spent beyond touching distance and leaves by infant were significantly more ($p<0.05$) in infant 5 in comparison to infant 2, despite being born to the same mother.

Table 37: Pairwise comparison of mother-infant relationship between male infants and female infants born to the same mother

Mother-infant interactions	Infant 1 and infant 4			Infant 2 and infant 5		
	U	W	p	U	W	p
Total time in contact	14.000	35.000	0.522	15.000	36.000	0.631
On nipple contact	14.000	35.000	0.522	12.000	33.000	0.337
Off nipple contact	14.000	35.000	0.522	12.000	33.000	0.337
Total time off contact	14.000	35.000	0.522	15.000	36.000	0.631
Touching distance	15.000	36.000	0.631	12.000	33.000	0.337
Beyond touching distance	15.000	36.000	0.631	57.500	193.500	0.023*
Total contact broken	15.000	36.000	0.631	11.000	32.000	0.262
Contact broken by mother	13.000	34.000	0.422	12.500	33.500	0.378
Contact broken by infant	7.000	28.000	0.036*	7.000	28.000	0.036*
Approach by mother	14.000	35.000	0.520	16.000	37.000	0.747
Approach by infant	5.000	26.000	0.037*	8.500	29.500	0.128

U=Mann-Whitney U, W=Wilcoxon W, p= Probability value

Allomothering behaviour

The care contact received by infants from non-mother females include grooming, touch hand, embrace, sitting-touching and touch genitals. In all the infants, grooming was the most frequent care received from non-mother females, followed by touch-hand, sitting touching, embrace and genital stimulation (figures 16-20). Spearman correlation test revealed no significant relationship between infants' age and the amount of care contact received, except for infant 2 (female), which shows positive correlation of embrace with age (Table 38).

Figure 16: Allomothering care received by infant 1

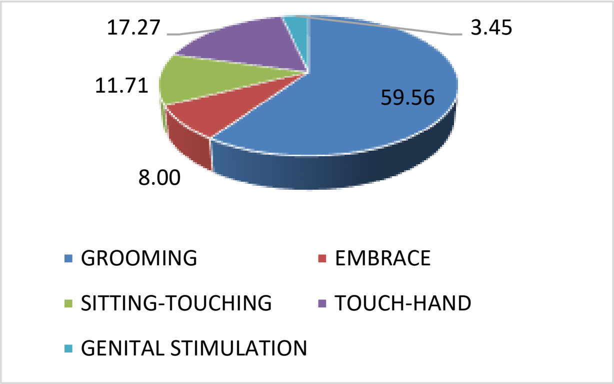


Figure 17: Allomothering care received by infant 2

Figure 18: Allomothering care received by infant 3

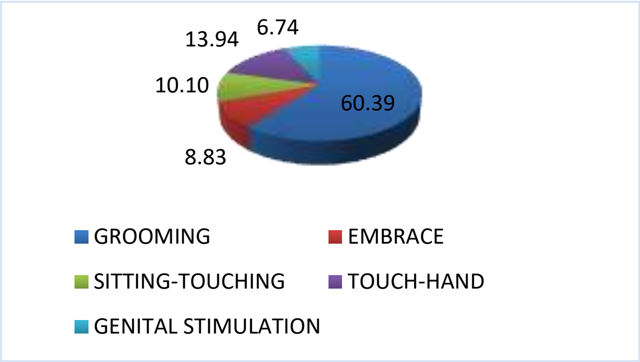


Figure 19: Allomothering care received by infant 5

Figure 20: Allomothering care received by infant 4

Table 38: Spearman correlation test between allomothering behaviour and age of infants (months)

Care contact		r_s	p value
Grooming	Infant 1	0.429	0.397
	Infant 2	0.086	0.872
	Infant 3	0.143	0.787
	Infant 4	-0.714	0.111
	Infant 5	-0.290	0.577
Embrace	Infant 1	-0.029	0.957
	Infant 2	0.829	0.042*
	Infant 3	-0.551	0.257
	Infant 4	0.088	0.868
	Infant 5	0.377	0.461
Sitting-touching	Infant 1	0.203	0.700
	Infant 2	-0.522	0.288
	Infant 3	0.086	0.872
	Infant 4	0.314	0.544
	Infant 5	-0.265	0.612
Touch-hand	Infant 1	0.486	0.329
	Infant 2	-0.486	0.329
	Infant 3	0.714	0.111
	Infant 4	0.6	0.208
	Infant 5	0.771	0.072
Touch-genitals	Infant 1	0.771	0.072
	Infant 2	-0.082	0.872
	Infant 3	0.667	0.148
	Infant 4	0.314	0.544
	Infant 5	0.377	0.461

r_s= Spearman correlation value, p= Probability value

Influence of infants' sex on allomothering behaviour

Mann-Whitney test revealed that female infants received significantly more care ($p<0.05$) from non-mother females than the male infants, except in the case of touch genitals (Table 39).

Table 39: Mann-Whitney test for allomothering care received and sex of the infants

Care contact	**U**	**W**	**P**
Grooming	0.000	171.000	0.001*
Embrace	28.500	199.500	0.001*
Sitting-touching	2.000	173.000	0.001*
Touch-hand	1.000	172.000	0.001*
Touch-genitals	106.000	184.000	0.932

U=Mann-Whitney U, W=Wilcoxon W, p= Probability value

DISCUSSIONS

Both in the wild and in captivity, primate mothers adjust their behaviour towards their infants according to sociodemographic conditions such as their age and experience, dominance rank, aggression received by them and their infants, sex of the infant, and size and composition of their group (Nash and Wheeler, 1982; Berman, 1984; Higley and Suomi, 1986; Nicolson, 1987, 1991). The present study reports both similarities and differences in mother-infant relationship observed from five mother-infant pairs of northern pigtailed macaque. The general course of development of the mother-infant relationship was similar in all mother-infant pairs. The total time on contact with mother and the time spent on nipple contact significantly decreased

($p<0.05$) with increase in infant's age. The total time off contact, time spent beyond touching distance of mother, total contact broken, leaves by mother and leaves by infant significantly increased ($p<0.05$) with increase in infant's age. The young primates undergo a long process of socialization through which they learn not only about the appropriate patterns of social behaviour but also about the minute aspects of their immediate environment (Poirier, 1972). The early stages of infant are characterized by close contact with and high degree of dependence on the mother. The infant then gradually develops towards more independence and its behaviour become increasingly environment-oriented. The mothers actively promote independence of the infant (Trivers, 1974). Newborns of most primate species can cling to their mother from birth (Dunbar and Badam, 1998), although the mother may help hold the infant in place when she walks or runs (Jay 1963; Blaffer and Hrdy, 1977). Krishna et al. (2008) observed on nipple contact to constitute about 70%of the total interactions within the first month of infant's life in lion-tailed macaque (*Macaca silenus*). Infants of stumptailed macaque also spent about 70% on nipple contact, reducing to slightly more than 10% in the sixth month (Solanki and Zothansiama, 2013), which is slightly less than the present finding. The study group of infants spent more than 80% of their time on nipple contact for the first month of the infant's life, which decreased to about 20% in the sixth month of the infant's life. In lion-tailed macaque, nipple contact decreased steeply up to the age of 5 months, accompanied by a sudden increase at about five and a half months (Krishna et al., 2008), this incidence was not observed in pigtailed macaque. The mothers of white-headed langur (*Trachypithecus leucocephalus*) also start regularly rejecting an infant from nursing at as early as five months of age (Zhao et al., 2008). The infants of lion-tailed macaque were observed to spent time more than 2 meters away from the mother at the age of three and a half months, approach and retrieve of infants by the mother was ended at the age of six months (Krishna et al., 2008). White-headed langur infants start to break contact at 4-5 weeks of age (Zhao et al., 2008). Captive red-shanked doucs (*Pygathrix nemaeus*), by the second month are able to move around on their own and frequently leave the mother (Yeong et al., 2010). At approximately 3-7 months of age, hanuman langurs (*Semnopithecus entellus*) are primarily moving around by themselves at will (Dunbar and Badam, 1998). This finding reflects the independence of the infants with time and increasing infant's age. In the present study, the infants attain independence towards the 6th month of the infant's life, even though the mother provides minor care beyond the 6th month, the major care and dependence of infants on the mother has ceased.

There was marked differences in regulation of mother-infant contact based on sex of the infant. The male infants were observed to show independence earlier than the female infants born to the same mothers. Maestripieri (2002) observed in pigtailed macaques (*Macaca nemestrina*) that female infants spent more time near their mothers and also spent longer time suckling than the male infants. Mothers also rejects suckling attempts by sons more than the attempts by female infants. More evident in the wild where competition for resources is higher, female infants may spend longer time closely to the mother because of the possible harassment by higher ranking members of the group (Dittus, 1979; Silk, 1983). Contrastingly, in lion-tailed macaques, on nipple contact by female infants ended earlier than the male infants. The female infants were also observed to be away from their mothers at longer distances earlier and more than the male infants, suggesting that female infants attained independence earlier than the male infants (Krishna et al., 2008). Parental investment has been shown to be sex biased in several primate species (Clark, 1978; Silk, 1983; Maestripieri, 2000). Parental investment theory predicts that parents should provide equal care to sons and daughters (Trivers, 1972). However, exceptions to this rule should occur only when offspring of one sex are more costly to produce or rear, therefore parents should invest more in the offspring sex which is less costly to rear, which provides the higher fitness returns, or both (Trivers and Willard, 1973; Trivers, 1985). Female infants have been reported to be more costly to rear than the male infants in several species of macaques (Simpson and Simpson, 1982; Paul and Thommen, 1984; Silk, 1988; van Schaik et al., 1989; Maestripieri, 2001). However, some reports noted the tendencies of mothers to display similar maternal styles with infants (Altmann, 1980; Goodall, 1986). Northern pigtailed macaque mothers who had two infants during the study period were observed to exhibit similar frequencies of maternal leave (leave by mother) and maternal approach (approach by mother). Therefore, the captive group of northern pigtailed macaque mothers provide equal care to their offsprings of both sexes. Since infants do not have more opportunity to move much away from the mother as compared to the natural situations, mother's attention maybe indifferent for male and female infants.

Alloparenting exists in many species of insects and birds, as well as some mammals (Wilson, 1971; Jarvis et al., 1994; Emlen, 1994; Asa, 1997; French, 1997; Garber and Leigh, 1997; Cockburn, 1998; Heinsohn and Double, 2004). Non-mother females play an important role in allomothering in certain primates (Emlen, 1991).

There are several reasons and hypothesis why allomothering occurs in mammals, it may be due to kin selection (helping related mothers in raising their offsprings). However, this does not explain infant handling among primates because they allomother infants from other groups also (Hardy, 1999; Silk, 1999; Maestripieri, 2007). In primates, it is likely that natural selection has favored this action to improve one's parenting skills. Allomothering was observed as soon as after 3 hours of birth in wild capped langur (Kumar et al., 2005). Among blue monkeys (*Cercopithercus* sp) and rhesus macaques (*Macaca mulatta*), the nulliparous females are the most active at allomothering (Rowell et al., 1964; Forster and Cords, 2005). In most species of primates, the juvenile females and adult females show more interest in infants than the males (Chamove et al., 1967; Rosenblum, 1972; Brandt and Mitchell, 1973; Gibber and Goy, 1985). Therefore, females, since a young age, may be biologically predisposed to behave nurturant towards infants (Nicolson, 1991). Further, Responsiveness theory states that female primates are strongly attracted to infants to make better mothers, because the more responsive the mother is, the higher the chance that she reacts better to her offsprings needs (Silk, 1999; Maestripieri, 2001; Silk et al., 2003b). It has been shown in vervet monkeys that the females who are more interested in infants as juveniles, are more likely to be successful in rearing their first infant (Fairbanks, 1990; Silk, 1999; Silk et al., 2003b; Maestripieri, 2007). It is also suggested that allomothering may play an important role in the socialization of the infant (Forster and Cords, 2005). In the present study, female infants received more allomothering care than the male infants, this may be due to the strong relationship and strong bond formation between the female primates. It may also reflect the protective nature of the females as harassment of female subordinates by the dominants has been reported in primates (Dittus, 1979; Silk, 1983).

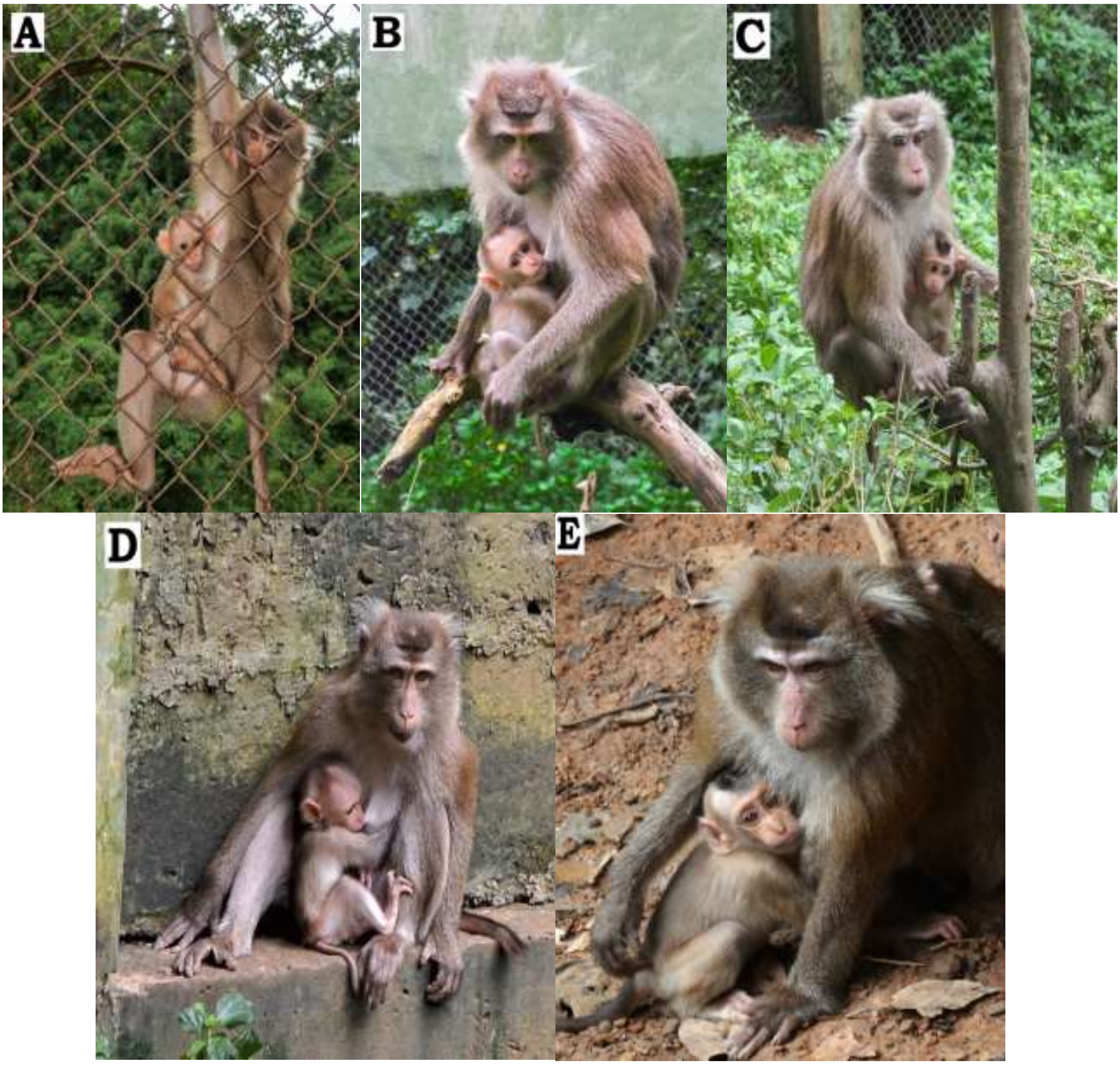


Photo plate 5: Mother-infant pairs of northern pigtailed macaques

A: Infant 1 (Nutei and her son)

B: Infant 2 (Luna and her daughter)

C: Infant 3 (Ginny and her son)

D: Infant 4 (Nutei and her daughter)

E: Infant 5 (Luna and her son)

CHAPTER X

REPRODUCTIVE BEHAVIOUR

INTRODUCTION

Several animals have preferences for certain mating partners (Paul, 2002). During the past few decades, it has become increasingly clear that neither competition over access to mates nor mate choice is restricted to one sex (Johnstone et al., 1996; Cunningham and Birkhead, 1998). Sexual selection theory predicts that female primates are the more selective sex for mate because of their parental investment and more reproductive investment (Trivers, 1972; Small, 1989). Males are less selective in their sexual preferences than females (Parga, 2006). Male mate selectivity may be expected only under certain conditions (Dewsburry, 1982). Male mate choice is expected to occur when females differ in quality and rank, when males seek long term partners or when they are constrained in their ability to mate with multiple females, or when they allocate resources to females or their offspring (Keddy, 1986; Cunningham and Birkhead, 1998). Competition among males for access to females is common in animals (Le Boeuf and Peterson, 1969; Clutton-Brock et al., 1982). Males of many species form clear dominance hierarchies (Smuts et al., 1987; Creel and Sands, 2003; Drea and Frank, 2003; Payne, 2003), and this dominance rank is usually positively correlated with mating success (Cowlishaw and Dunbar, 1991; Packer et al., 1991; Bercovitch, 1992a, 1992b; Dunbar and Cowlishaw, 1992; Mainguy et al., 2008; Rodriguez-Llanes et al., 2009). Males competing for mating access to females will use mating tactics, i.e., distinct behavioural patterns leading to mating access, depending on their age, health, and body condition (Bercovitch and Nürnberg, 1996; Bercovitch, 1997) and on their competitors' characteristics (Andersson, 1994). For example, strong or dominant males can gain mating access based on their fighting abilities or dominance rank (Cowlishaw and Dunbar, 1991; Mainguy et al., 2008; Spong et al., 2008), whereas subordinate males may use other tactics (Smuts, 1985; Noë and Sluijter, 1990; Apollonia et al., 1992; Mainguy et al., 2008). However, male mating success depends not only on the behaviour of other males, but also on female mating tactics (Trivers, 1972). Females may prefer particular males (Andersson, 1994) or mate with many males (Richard, 1992; Widdiget al., 2004; Nikitopoulos et al., 2005), allowing mating access for multiple males that employ different tactics. Dominant males ensure their mating access and success by possessive following of females and by disrupting consorts of females with other males (Berard et al., 1994). It has been reported in various studies that subordinate males have more mating access to females than predicted: savanna baboons (Bercovitch, 1986; Noë and Sluijter, 1990), olive baboons (Smuts, 1985), and rhesus macaques (*Macaca mulatta*: Dubuc et al., 2011). This suggests that subordinate males employ other tactics or that females have other preferences (Alberts et al., 2003). Especially in primates, where multiple males can live in the same group with multiple mating females (Dixson, 1997), there are ample opportunities for males to employ different mating tactics. Middle-ranking males may form coalitions against high-ranking males to gain access to females, e.g., revolutionary coalitions (Noë and Sluijter, 1990; Alberts et al., 2003; van Schaik et al., 2004), or hide their mating and mate quickly (Berard et al., 1994). Alternatively, male affiliation with females may lead to mating opportunities, and developing bonds with females may be very important for individual males to attain mating opportunities (Alberts et al., 2003). Low-ranking males may entice females to mate with them by providing them with benefits. Grooming is a service that can be interchanged against other benefits, including mating opportunities (Barrett and Henzi, 2006). Males groom females and may mount these females subsequently, e.g., bonnet macaques (*Macaca radiata*: Kurup, 1988), chimpanzees (Hemelrijk et al., 1992), and long-tailed macaques (*Macaca fascicularis*: Gumert, 2000). This indicates that male grooming may constitute a mating strategy that males employ in the short term, i.e., a biological market for mating behaviour (Noë et al., 1991) or long term, i.e., good social relationships (Smuts, 1985) to gain access to females. Therefore, males will groom females in exchange for mating access and females will reciprocate by allowing mating access. Mating opportunities with high-ranking females have a greater value because high-ranking females produce more offspring with better survivorship chances compared to low ranking females (long-tailed macaques: Noordwijk and Schaik, 1999). High ranking males may provide better genetic, i.e., direct, benefits to a female or be better able to force a female to mate than low-ranking males (Gumert, 2007). In addition, a more dominant male may provide better protection, i.e., indirect benefits, for the female and her future offspring (Smuts, 1985). Females may benefit from employing a dual strategy of both "honest" and "dishonest" sexual behaviours, particularly in a promiscuous mating system (Nunn, 1999; Stumpf and Boesch, 2005). By mating with several males, females may confuse paternity, thus reducing the incentive for males to commit infanticide (Hrdy, 1979). Paternity confusion may also increase the likelihood that males will provide direct benefits to females or their offspring, such as infant carrying, tolerance during feeding, and protection from infanticide and/or predation (Deag, 1980; Crockett and Sekulic, 1984; van Schaik, 1994; Borries et al., 1999). On the other hand, it may also be advantageous for females to advertise fertility when the likelihood of conception is high in order to increase the chances of mating and conceiving with the highest quality male (Maynard Smith, 1991; Clutton-Brock and McAuliffe, 2009). Indeed, in several primate species, females were found to direct proceptive and receptive behaviours more frequently toward high-ranking males (Stumpf and Boesch, 2005; Barelli et al., 2008; Knott et al., 2010)

MATERIALS AND METHODS

Subjects

Study of reproductive behaviour was done on 7 adult pigtailed macaques. The study group comprises of 3 adult males- alpha male, beta male and gamma male; and 4 adult females (Adult female 1- named Nutei, adult female 2- named Luna, adult female 3-named Ginny, adult female 4- named Tonks). No definite linear hierarchy was observed among the adult females.

Data collection

The study was carried out during one breeding season (6 months) on October, 2016 to March, 2017. Observations were recorded by focal sampling and sampling all occurrence method (Altmann, 1974) throughout the breeding season for sexual activities like sexual solicitations, copulation, approach, grooming, female present, female look back. The frequencies of female approach to males, present to males, grooming and look back are used as the indices of female sexual proceptivity. The frequencies of male approach to female and grooming are used as indices of female sexual attractivity or male courtship behaviour towards females. The different acts and postures during mating are described below.

Copulation: A male mounts over a female with intromission and thrusting, with or without ejaculation.

Successful copulation- mounting with vaginal intromission and thrusting terminated with ejaculation.

Unsuccessful copulation- mounting with vaginal intromission and thrusting terminated without ejaculation.

Approach: When animal moves close to another animal within 1 m distance (Soltis, 1999).

Groom: The cleaning or combing through the pelage of another organism with hands (Kurland, 1977).

Look back: a female looks over her shoulder at a male while being within 3m of him without showing submission (Enomoto, 1974).

Present: Female orients her perineal region towards males within 1m of proximity (Enomto, 1974).

Data analysis

Pearson correlation test was performed to determine whether the number of successful copulations correlated with the number of interactions between males and females, age of females, female sexual proceptivity and female sexual attractivity. Kruskal-Wallis test was employed to determine the differing levels of female monopolization by males. The significant differing levels of female monopolization as indicated by Kruskal-Wallis test were subjected to pair-wise comparison by using Mann-Whitney test. All the statistical analysis was performed using SPSS version 18.0.

RESULTS

The incidence of sexual interactions between each adult male (alpha male, beta male and gamma male) and the adult females are given on table 40, table 41 and table 42 respectively. The frequencies of female sexual proceptivity and the male courtship behaviour in one-hour time interval are given on figure 21 and 22 respectively.

Table 40: Sexual interactions between alpha male and adult females

	Nutei	Luna	Ginny	Tonks
Female Sexual Proceptivity				
Approach by female/hour	11.5	12.5	9.5	13.5
Present/hour	17.5	12.5	9	7.5
Groom by female (min/hour)	6.7	9.1	10.8	8.5
Look back/hour	4	7	9	5
Female Sexual Attractivity				
Approach by male/hour	21.5	18.5	15.5	11.5
Groom by male (min/hour)	16.5	12.2	8.1	7.5
Unsuccessful copulation	2	2	1	3
Successful copulation	11	9	6	4

Table 41: Sexual interactions between beta male and adult females

	Nutei	Luna	Ginny	Tonks
Female Sexual Proceptivity				
Approach by female/hour	9.5	12.5	5.5	5.1
Present/hour	8.2	5.5	12.5	6.5
Groom by female (min/hour)	5.1	6.2	4.2	7.5
Look back/hour	4	7	2	8
Female Sexual Attractivity				
Approach by male/hour	9.5	7.3	15.2	6.9
Groom by male (min/hour)	8.9	4.2	12.5	5.1
Unsuccessful copulation	1	2	2	1
Successful copulation	4	2	1	2

Table 42: Sexual interactions between gamma male and adult females

Female Sexual Proceptivity	Nutei	Luna	Ginny	Tonks
Approach by female/hour	5.2	3.8	8.5	6.5
Present/hour	8.5	4.2	6.5	3.8
Groom by female (min/hour)	5.5	6.5	3.5	3.2
Look back/hour	3	5	2	6
Female Sexual Attractivity				
Approach by male/hour	10.5	4.2	6.5	4.1
Groom by male (min/hour)	11.5	3.2	6.2	3.5
Unsuccessful copulation	3	0	2	1
Successful copulation	1	2	3	2

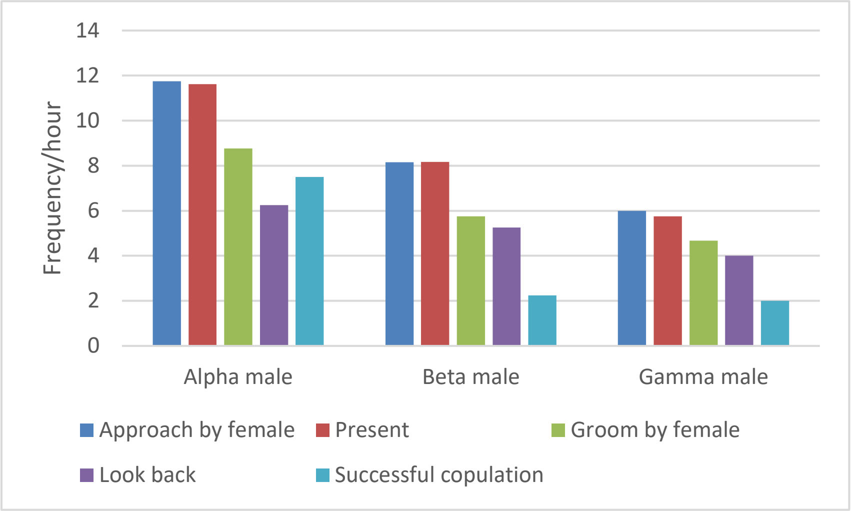


Figure 21: Female sexual proceptivity towards the adult males and mating success

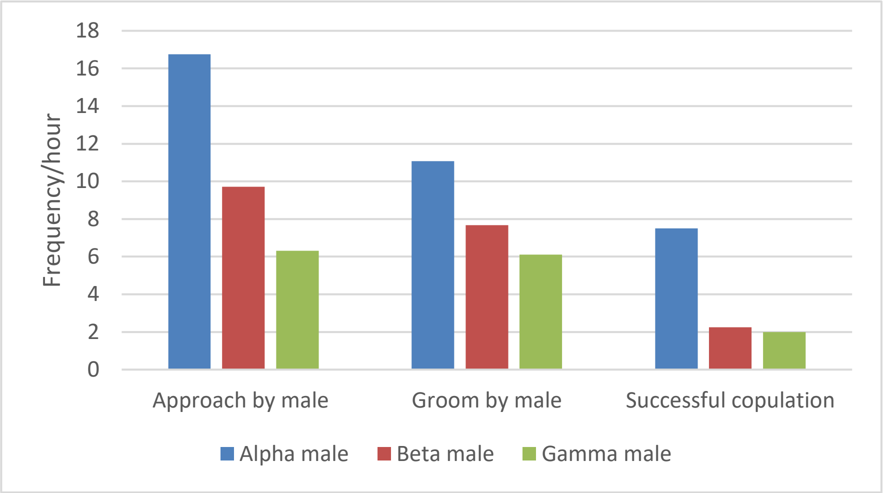


Figure 22: Male courtship behaviour towards the adult females and mating success

Successful copulation

Pearson correlation test showed no significant correlation of the number of successful copulation and the total number of interactions between adult males and adult females (Alpha male: Pearson P=0.966, p=0.166; Beta male: Pearson P=0.272, p=0.825; Gamma male: Pearson P=0.993, p=0.073). The age of the females also did not influence the number of successful copulations (Alpha male: Pearson P=0.028, p=0.947; Beta male: Pearson P=-0.040, p=0.925; Gamma male: Pearson P=0.593, p=0.121). Pearson correlation test (Table 43) revealed no significant correlation ($p>0.05$) between the number of successful copulation and the amount of approach by females, groom by females and look back by females. However, the number of successful copulations was found to be positively correlated ($p<0.05$) with the number of females present to males, the frequencies of approach and grooming received by the females from the males. The more the females displayed the present gesture to males, the more males approach and groom the females, the more it leads to successful copulation.

Kruskal-Wallis test revealed significant variation on the amount of successful copulation between the females and the different males during the study ($\chi^2=7.256$, df=2, p=0.027*). Mann-Whitney pairwise test further revealed the occurrence of successful copulation to be higher between the females with the alpha male than with the beta male (U=0.500, W=10.500, p=0.028*), and the gamma male (U=0.000, W=10.000, p=0.020*). However, the successful copulation of females by beta and gamma males do not differ significantly (U=7.500, W=17.500, p=0.877).

Table 43: Pearson correlation test for successful copulation and females sexual proceptivity and male courtship behaviour

	Alpha male		Beta male		Gamma male	
Activities	Pearson P	p value	Pearson P	P value	Pearson P	P value
Approach by female	0.569	0.614	0.302	0.805	0.272	0.825
Present by female	0.972	0.028*	0.998	0.040*	1.000	0.015*
Groom by female	0.707	0.500	0.224	0.856	0.636	0.561
Look back	0.963	0.173	0.669	0.534	0.993	0.073
Approach by male	0.991	0.009*	0.998	0.002*	0.976	0.024*
Groom by male	0.966	0.034*	0.972	0.028*	0.999	0.030*

Females sexual proceptivity

Kruskal-Wallis test revealed significant differences on the proceptivity levels of females towards the different males, except in the case of look back. (Approach: $\chi2=7.200$, p=0.027; Present: $\chi2=9.582$, p=0.008; Grooming: $\chi2=7.261$, p=0.027; Look back: : $\chi2=0.487$, p=0.784). Pairwise comparison using Mann-Whitney test (Table 44) revealed that alpha male dominates over beta male and gamma male for the female sexual proceptivities, and beta male dominates over gamma male. The females approach, groom and present themselves to alpha males more than the beta male and the gamma male, and in turn more to the beta male than the gamma male. However, the lookback gesture was shown to the adult males more or less the same amounts.

Table 44: Mann-Whitney test for the pairwise comparison of females proceptivity towards different males

Activities	Pair	U	W	p
Approach	Alpha male vs beta male	0.000	6.000	0.048*
	Alpha male vs gamma male	0.000	171.000	0.001*
	Beta male vs gamma male	288.500	753.500	0.017*
Present	Alpha male vs beta male	297.000	762.000	0.023*
	Alpha male vs gamma male	276.000	741.000	0.010*
	Beta male vs gamma male	279.000	744.000	0.011*
Grooming	Alpha male vs beta male	0.000	10.000	0.029*
	Alpha male vs gamma male	5.000	33.000	0.012*
	Beta male vs gamma male	0.000	6.000	0.046*

U=Mann-Whitney U, W=Wilcoxon W, p= Probability value

Male courtship behaviour

Kruskal-Wallis test conducted for the differing levels of male courtship behaviour between the different males were found to be statistically significant in case of approach ($\chi2$=7.654, p=0.022), but not for grooming ($\chi2$=3.115, p=0.211). The pairwise test of Mann-Whitney (Table 45) revealed the dominance of alpha male over beta male and gamma male, but no significant variation was found between beta male and gamma male. (Alpha male vs Beta male: U=1.000, W=11.000, p=0.043; Alpha male vs Gamma male: U=0.500, W=10.500, p=0.029; Beta male vs Gamma male: U=3.000, W=13.000, p=0.149). Alpha male approaches the adult females more than beta male and gamma male. But the time spent on grooming the adult females by alpha male, beta male and gamma male did not differ.

Table 45: Mann-Whitney test for the pairwise comparison of females proceptivity towards different males

Activities	Pair of adult males	U	W	p
Approach	Alpha male vs beta male	1.000	11.000	0.043*
	Alpha male vs gamma male	0.500	10.500	0.029*
	Beta male vs gamma male	3.000	13.000	0.149

U=Mann-Whitney U, W=Wilcoxon W, p= Probability value

DISCUSSIONS

In the present study, the occurrence of successful copulation was found to be higher between the females with the alpha male than with the beta male and the gamma male. However, the successful mating of females by beta and gamma males do not differ significantly. Previous researches have shown that dominance striving among male primates, like other animals, generally results in increased reproductive success. Evidence has shown positive correlation between dominance rank and mating or reproductive success in many species of primates (Cowlishaw and Dunbar, 1991; Bercovitch, 1992a, 1992b; Dunbar and Cowlishaw, 1992; Berard et al., 1994; de Ruiter et al., 1994; Zothansiama et al., 2014). The present study provides evidence, that dominant males have higher reproductive success in captive northern pigtailed macaque. The number of successful copulations was also found to be positively correlated with the number of females present to males, the frequencies of approach and grooming received by the females from the males. The more the females displayed the present gesture to males, the more males approach and groom the females, the more it leads to successful copulation. Similar to these findings, male grooming has been found to increase in successful mating (Soltis, 1999; Manson, 1996). During the mating season, both males and females of rhesus macaques groomed the opposite sex more often suggesting that grooming in the mating context may enhance access to mating partners. Also, there was a clear relation between male grooming and mating access (Massen et al., 2012). The study in stumptailed macaques (Zothansiama and Solanki, 2015) reported grooming as an important mating strategy where all the adult males groomed the females prior to copulation. In addition, females that received more successful copulation were found to received more grooming from the adult males in every sub-group. Grooming is a service that can be interchanged against other benefits, including mating opportunity (Barrett and Henzi, 2006). Generally, male grooms receptive females and may mount these females subsequently (Kurup, 1988; Gumert, 2000). Male aggression preceded female approaches to the male, indicating that these males may have coerced the females to remain with them. Male grooming of the female and prolonged mount series also may be mate-guarding tactics (Manson, 1996; Soltis, 1999). Thus, high-ranking male strategies may have prevailed over those of females in part because of successful mate guarding and coercion. Females were often seen attempting to escape from dominant males with whom they were mating. Moreover, rhesus macaque females show marked multiple mating (Lindburg, 1971), suggesting that female choice may play a role in male and female mating access and success.

Previous reviewers of mate choice in nonhuman primates revealed that females of many species not only actively solicit sexual interactions with males but also often display clear preferences for certain males and reject solicitations of others (Smuts, 1987; Small, 1989; Keddy- Hector, 1992; Manson, 1995; Dixson, 1998), suggesting that female choice is a potentially powerful selective force among nonhuman primates (Manson, 1992). In many primates, females copulate with more than one male during a single mating season (Dixson, 1997; 1998). In the present study, the females were found to show higher proceptivity levels to the alpha male more than the beta male. The females approach, groom and present themselves to alpha male more than the beta male and the gamma male, and in turn more to the gamma male than the beta male. However, the lookback gesture was shown to the adult males more or less the same amounts. Additionally, the alpha male also shows more courtship behaviour towards the females more than the beta male and the gamma males. The findings are consistent with the studies on a variety of other primates where females exhibited more interest in the dominant male for mating (Manson, 1992; Dixon et al., 1993; Altmann et al., 1996; Bercovitch and Nurnberg, 1997; Gust et al., 1998; van Schaik et al., 2000; Borries et al., 2001; Heistermann et al., 2001; Pazol, 2003; Carnegie et al., 2004, 2005; Engelhardt et al., 2005, 2007; Arlet et al., 2007; Brauch et al., 2007). This interest was reflected in both higher rates of proceptivity and higher rates of mating (receptivity). Moreover, females were more aggressive toward the adolescent males even if the adult male was nowhere in sight. The observed more preference for dominant male over the subordinate males can be seen as the range of solutions for the female's dilemma; she could choose to mate the best male, capable to protect her and her offspring (van Schaik et al., 2000). Females mating mainly with high-ranking males can be explained by monopolization by these males (mate guarding), their aggression towards females, female preference based on 'the best male' or infant care. Physically superior and dominant males are most capable of protecting their offspring against infanticidal attacks, and novel males or males that rise in the dominance hierarchy are most likely to commit infanticide.

Females may also choose to mate with mid-ranking males (*Macaca mulatta*: Manson, 1992, 1994b; *Macaca fuscata*: Huffman, 1991; Soltis et al., 2001). Smith (1994) reported that captive group of rhesus macaques females did not prefer top-ranking males but potential younger ones that would ultimately achieve top rank. The rank order in adult male has no significant correlation with mating activity in Japanese monkey. Female decides whether to allow mating to take place; mere dominance may be insufficient to attract the estrous female (Takahata, 1982). In Papionines, there are several participations of females in 'sneak' matings with subordinate males (Smuts, 1983; Nunn, 1999). In baboons, females mate with multiple males but may try to develop a special relationship with at least one male (Smuts, 1985), who also forms an affiliative bond with her offspring (Bercovitch, 1991; Palombit et al., 1997). Females may show preferences towards newcomer males (Small, 1989; Bercovitch, 1997). The females of Japanese macaque frequently reject courtship solicitations from higher ranking males (Enomoto, 1978; Takahata, 1982). The females draw many advantages with multiple male mating (Parker, 1984; Soltis, 2002) including infanticide avoidance by confusing paternity, inbreeding avoidance and gaining access to resources such as food in the territory of extra-unit males (Guo, 2008). Even in species characterized by negligible male parental care, such as vervets, females appear to prefer males that direct friendly behaviours toward their, and other females' offspring (Keddy-Hector, 1992). Moreover, males that did not mate with an infant's mother appear to be more likely to commit infanticide (Soltis et al., 2000), while putative or actual fathers are much more likely to protect their offspring from infanticidal males (Borries et al., 1999). Clearly, any behaviour that lowers the risk of infanticide would be highly beneficial for female primates vulnerable to infanticide, and several of their mate choice decisions appear to be consistent with this interpretation. Additionally, males may have used coercion and mate guarding to prevent females from mating with multiple males (Smuts and Smuts, 1993). High ranking males mostly contain elements of male coercion and mate guarding, and remain associated with mating success (Soltis, 1999). Females of some primates preferred males of various dominance ranks, but were monopolized by dominant males, which prevented them from mating with mid- and low-ranking males (Manson, 1992, 1994a, 1994b; Soltis et al., 2001). In this study, the male dominance rank plays an important role in the mating access, high ranking males mated with more females and more often than low ranking ones.

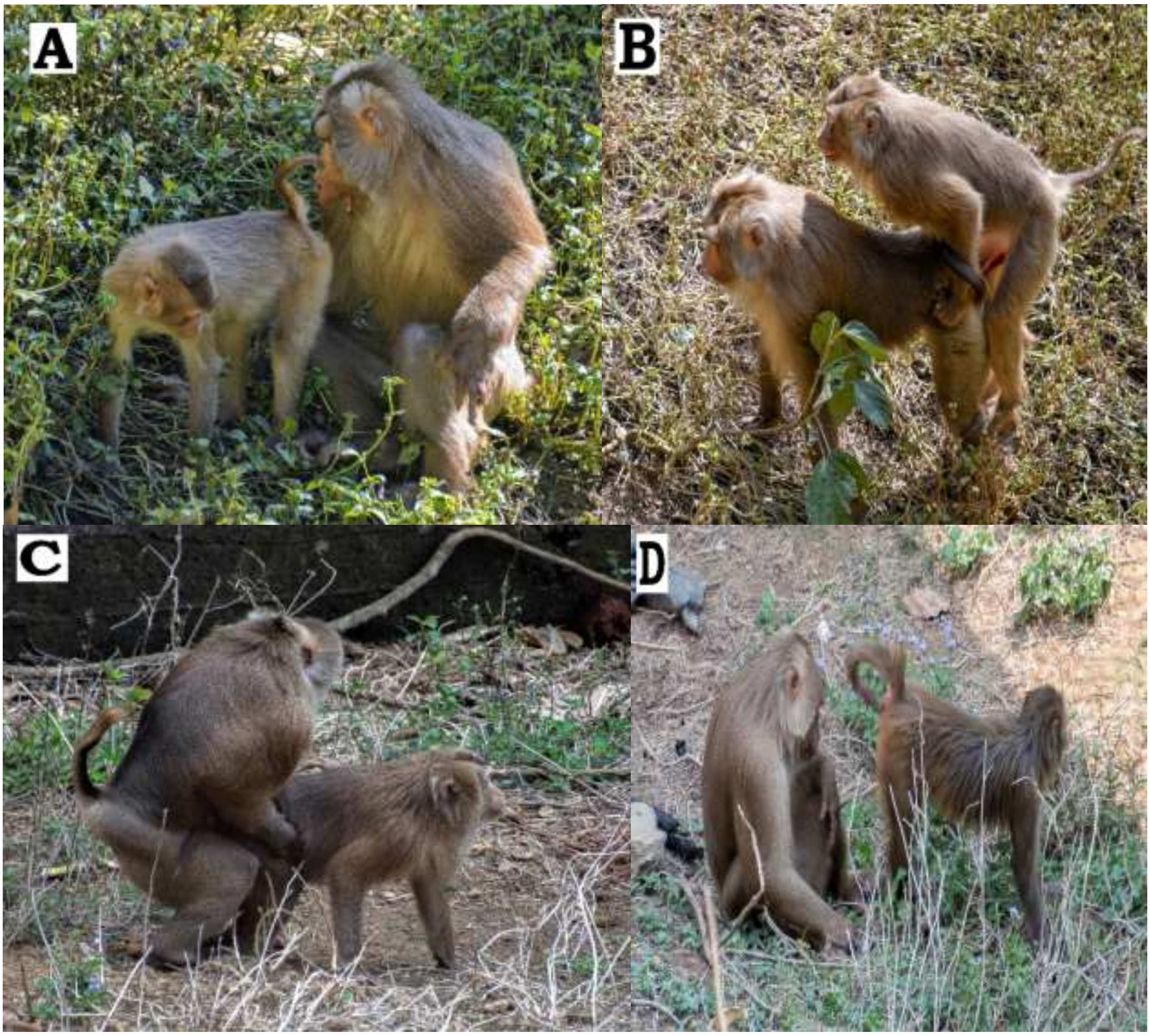


Photo plate 6: Mating behaviour in northern pigtailed macaques

A: Tonks (Adult female 4) presents to beta male

B: Adult female 2 (Luna) mounted by gamma male

C: Adult female 1 (Nutei) mounted by alpha male

D: Adult female presenting to alpha male

CHAPTER XI

MOLECULAR CHARACTERIZATION THROUGH DNA BARCODE

INTRODUCTION

The identification and characterization of living things are fundamental to biological science. Taxonomy based on morphological analyses can be problematic due to either convergence in phenotype among unrelated species or the failure to identify 'cryptic species' where morphologic divergence has not kept pace with genetic divergence. (Lorenz et al., 2005). In an effort to standardize the approach to species identification using molecular techniques, it has been proposed that as many species as possible be characterized for the same genetic markers (Blaxter, 2004). Analysis of molecular data has proven to be important for understanding deep phylogenetic relationships (Blair and Hedges, 2005; Regier et al., 2005), examining population structure within a species (Avise et al., 1987; Zhang and Hewitt, 2003), assigning unknown specimens or immatures to reference species (Olson, 1991; Bartlett and Davidson, 1992; Sperling et al., 1994; Hebert et al., 2003), and diagnosing and delimiting cryptic species (Sperling and Hickey, 1994; Goetze, 2003; Hebert et al., 2003; Scheffer et al., 2004; Blair et al., 2005; Hendrixson and Bond, 2005). The use of molecular characters for identification of unknowns has proven to be useful and highly effective, and can be achieved using only a small number of molecular differences (Sperling and Hickey, 1994; Wells et al., 2001; Hebert et al., 2003). However, delimiting species ideally requires data from many different sources, such as morphology, behaviour, and multiple molecular markers (Funk and Omland, 2003; Dayrat, 2005). Mitochondrial DNA (mtDNA) genes have long dominated the field of molecular systematics because of their maternal inheritance, limited recombination, rapid evolution, and the robustness of mtDNA against degradation, making them ideal markers for many species-level questions (Avise et al., 1987).

For the goals of species identification in animals, the cytochrome c oxidase subunit 1 (cox1) has been introduced as standard marker. Cox1 could serve as a fast and accurate marker for the identification of animal species, and for the discovery of new species across the tree of life (Hebert et al., 2003). One of the key features of the DNA barcoding project, as proposed by Hebert et al. (2003), is the designation of a single mtDNA fragment at the 50 end of cytochrome coxidase I (COI) gene to act as a 'barcode' to identify and delineate all animal life. A DNA barcode is a short sequence of nucleotides taken from an appropriate part of an organism's genome that is used to identify it at species level. Intraspecific variation in this gene is generally <10% of that observed between species. Moreover, insertions and deletions are rare (Blaxter, 2004). DNA barcoding was employed to resolve species ambiguity within narrow taxonomic groupings in some of the studies (Brown et al., 2003; Hebert et.al., 2004; Hogg and Hebert, 2004; Paquin and Hedin, 2004; Penton et al., 2004; Whiteman et al., 2004; Barrett and Hebert, 2005; Greenstone et al., 2005; Lambert et al., 2005; Vences et al., 2005; Ward et al., 2005; Hajibabaei et al., 2006; Smith et al., 2006b) or to identify higher taxa from wider assemblages of animals in others (Hebert et al., 2003; Remigio and Hebert, 2003; Barrett and Hebert, 2005). In addition, DNA-sequencing technology has been used for identifying organisms from other Kingdoms including plants (Kress et al., 2005; Shaw et al., 2005), bacteria (Woese, 1987; Unwin and Maiden, 2003), protists (Cavalier, 2004) and viruses (Mayo and Horzinek, 1998).

The use of DNA barcodes in primatology has been explored by Hajibabaei et al. (2006) and Lorenz et al. (2005). Numerous DNA barcoding studies conducted thus revealed that more than 90% of species under study could be identified by this method. For example, Clare et al. (2007) for Guyanan bats were able to allocate 93% of their species correctly, and Kerr et al. (2007) for North American breeding bird species rightly classified 95% of all species. Primates are a target group for scientific and technological development due to their importance in biomedicine and conservation biology, especially given their evolutionary proximity to humans. Within this context, the collection and storage of primate resources covering all branches of their taxonomy is an urgent need to boost primate molecular biology. Lorenz et al. (2005) generated molecular bar code for 56 species of primates using 'universal cox 1 primers' and primers developed specifically for the primate taxa. Of the 56 species, only *Macaca mulatta* is included among the eight species that are found in Mizoram. DNA barcoding and the evolutionary study of primates in Mizoram has not been done. This study was carried out to confirm the identification of the macaques in Mizoram, and to provide knowledge for better understanding of the phylogeny of macaques in Mizoram.

MATERIALS AND METHODS

Sample collection

Fecal samples were collected from one individual each of the four macaques i.e., rhesus macaque (*Macaca mullata),* assamese macaque *(Macaca assamensis),* stumptailed macaque *(Macaca arctoides)* and northern pigtailed macaque *(Macaca leonina).* DNA extraction was done using QIAmp Stool Kit (QIAGEN). However, proper genomic DNA was not isolated from the stool samples even after several attempts. Therefore, study on the molecular aspects was done using blood samples. Blood samples were collected from adult captive animals at Aizawl Zoological Park, Sakawrtuichhun by a veterinary doctor at the Zoo, with the permission of The Chief Wildlife Warden, Department of Environment, Forest and Climate Change under the permission number A.33011/4/2011-CWLW/Vol.II/388-89. Blood samples (0.5 ml) were taken intravenously from the four macaques in EDTA vials. The blood samples were stored at -20°C till further processing.

DNA extraction and amplification

DNA extraction was done using phenol-chloroform method (Ghatak et al., 2013). 50 µL of blood sample was taken in a 1.5 ml tube and 300µL of RBC lysis buffer was added. The mixture was vortexed for 5 minutes and spun for 5 minutes. Centrifugation was done at 5000rpm for 10 minutes at 4˚C and the supernatant was discarded. The steps were repeated and 500 µL of 1X PBS Buffer was added to the tubes. The tubes were vortexed for 5 minutes. The tubes were spanned for 5 minutes and were centrifuged at 5000rpm 10 minutes at 4˚C. The supernatants were discarded. These steps were repeated. 300µL of extraction buffer was added. 50µL of proteinase K was added. The tubes were incubated at 56 ˚C for 1 hour or overnight. The tubes were shaken vigorously. 500 µL of PCI and mixed well. The tubes were centrifuged at 5000 rpm for 10 minutes. The upper part of the samples were taken out and were put to another tube and the lower part was discarded. 500 µL of chilled isopropanol was added. 60 µL of sodium acetate was added and the tubes were incubated at -20 ˚C for 1 hour. The tubes were centrifuged at 12000rpm for 10 minutes. The supernatant was discarded and the tubes were allowed to dry for about

20-30 minutes. 30 µL of nuclease free water was added and the tubes were tapped vigorously to mix.

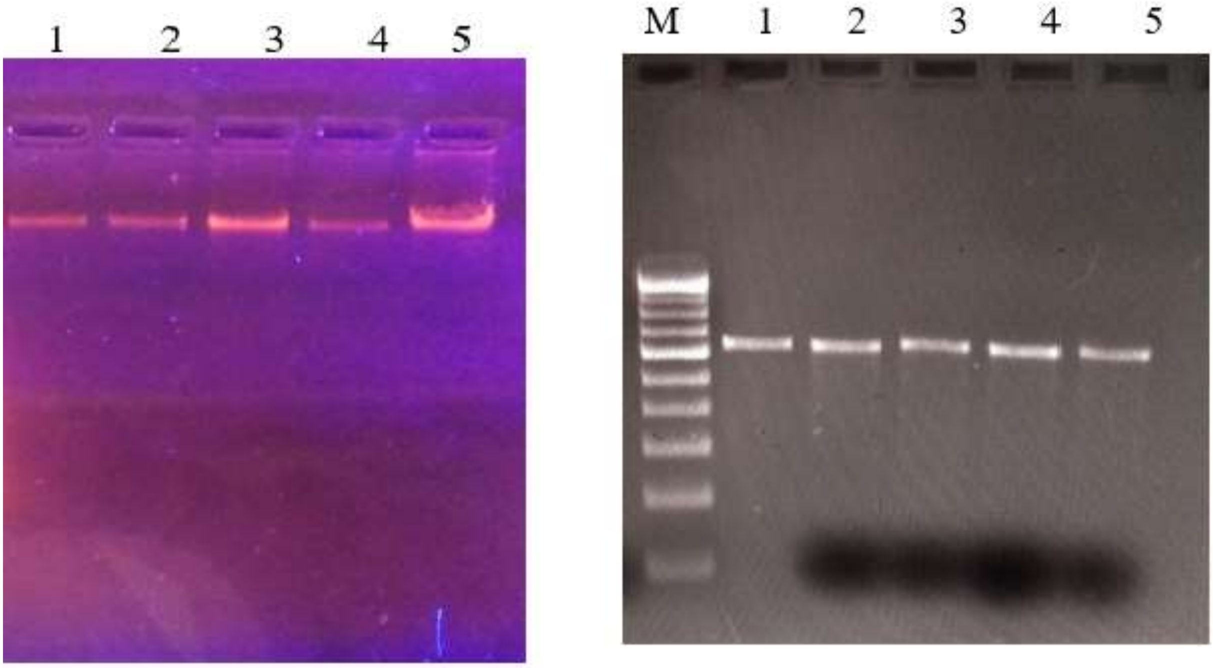
PCR reactions were done in a total volume of 25 µl and consisted of 2.5 µL of 10X PCR buffer, 2.5 µL of 25 mM MgCl2, 2.0 µL of 10mM dNTP mix, 0.2 µL of each primer and 0.2µL of Taq DNA polymerase, 2.0 µL of DNA template and H_2O to make it to 25 µL. The thermocycling conditions were as follows: 96℃ for 10 min to activate the Taq polymerase and then 35 cycles of 96 ℃ for 1 min, 54℃ for 1 min and 72 ℃ for 1 min followed by a final hold of 72℃ for 10 min. The PCR products were visualized in 2% agarose gel and were purified using QIA quick PCR purification kit (Qiagen, Germany). Sanger sequencing was done at the Department of Biotechnology, Mizoram University.

Sequence analysis

The forward and reverse sequence files for each sample were analysed and a consensus sequence for each sample was created using 'Sequencher'. The consensus sequences were in turn aligned using Clustalx of MEGA and exported into a NEXUS file for analysis in BEAST. In order to validate the cox1 sequences obtained, comparison was done with cox1 sequences from primate species for which the whole mitochondrial DNA sequence has been established and the partial sequences which were deposited to GenBank. The sequences were used to construct a phylogenetic tree of macaques using BEAST (Bayesian Evolutionary Analysis Sampling Trees) software.

RESULTS

A region approximately 700bp long near the 5’ terminus of the cox1 gene was amplified. All the samples in the study were successfully amplified (Figure 23). The sequences were subjected to NCBI BLAST for confirmation and were submitted to GenBank with accession numbers- MH350401 (*Macaca arctoides)*, MH350402 (*Macaca assamensis),* MH350403 (*Macaca leonina),* MH350404 (*Macaca mulatta).* The phylogenetic tree constructed (figure 24) suggests that macaques evolved ~5.72 million years ago, and that the macaques of Mizoram are closely related to the macaques of Myanmar.



A **B**

Figure 23: Agarose gel photos

A: Genomic DNA extracted from blood samples (*1-Macaca arctoides,* 2- *Macaca leonina,* 3-*Macaca mulatta*, 4-*Macaca mulatta*, 5-*Macaca assamensis)*

B: Amplified cox1 gene in PCR (1-*Macaca leonina,* 2-*Macaca mulatta*, 3-*Macaca mulatta*, 4-*Macaca assamensis*, 5-*Macaca arctoides)*

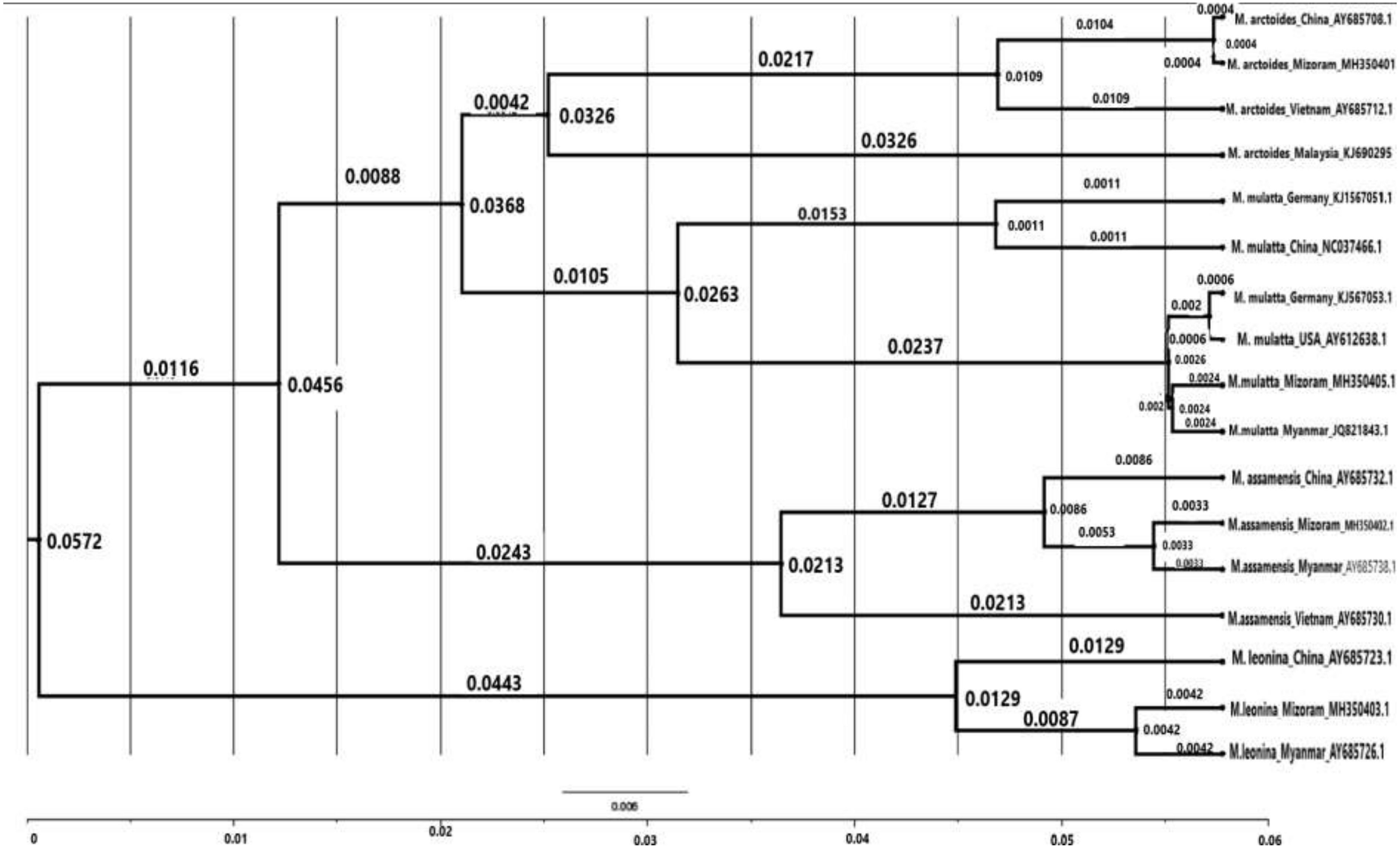


Figure 24: Phylogenetic tree constructed from the cox1 sequences of macaques

DISCUSSIONS

This study indicates that the macaques of Mizoram are closely related to the macaques of Myanmar. NCBI BLAST also revealed highest cox1 sequence similarities of the macaques of Mizoram with the macaques found in Myanmar. These high similarities can be explained by the geographical closeness of Myanmar and Mizoram. Migration from Myanmar to Mizoram could have taken place, since the two regions are adjacent to each other. The present study also revealed the emergence of macaques to be around 5.72 million years ago, which is in accordance with the study conducted by previous researchers (Delson, 1980, 1996; Tosi et al., 2000; Lorenz et al., 2005; Ziegler et al., 2007; Perelman et al., 2011). Current views on primate taxononomy concur that 67–69 primate genera originated from a common ancestor during the Cretaceous/Paleocene boundary roughly 80–90 million years ago (Goodman et al., 1998; Groves, 2001; Wilson and Reeder, 2005). An Eocene expansion formed the major extant lineages of 1) Strepsirrhini, which is composed of Lorisiformes (galagos, pottos, lorises), Chiromyiformes (Malagasy aye-aye) and Lemuriformes (Malagasy lemurs); 2) Tarsiiformes (tarsiers) and 3) Simiiformes composed of Platyrrhini (New World monkeys) and Catarrhini, which includes Cercopithecoidea (Old World monkeys) and Hominoidea (human, great apes, gibbons). Primate taxonomy, initially imputed from morphological, adaptive, bio-geographical, reproductive and behavioural traits, with inferences from the fossil record is complex (Goodman et al., 1998; Groves, 2001; Wilson and Reeder, 2005).

Macaques (genus *Macaca*) represent one of the major lineages of the family Cercopithecidae (Old World monkeys). This family is the only extant family in the superfamily Cercopithecoidea, which, combined with the superfamily Hominoidea (human and apes), constitutes the infraorder Catarrhini. Accordingly, besides great apes (chimpanzees, gorillas, orangutans) and small apes (gibbons), Old World monkeys are human's closest living relatives. According to fossil remains and genetic data, both superfamilies diverged about 32 million years ago (Perelman et al., 2011; Springer et al., 2012; Finstermeier, 2013; Pozzi et al., 2014). In contrast, marmosets, squirrel monkeys, and owl monkeys are representatives of the infraorder Platyrrhini (New World monkeys) and diverged from Catarrhini 46 million years ago (Perelman et al., 2011; Springer et al., 2012; Finstermeier, 2013; Pozzi et al., 2014). Old World monkeys are not only phylogenetically more closely related to humans than New World monkeys, they are also more similar to human in physiology, anatomy, genetics, immune response, and behaviour (Whitehead and Jolly, 2000; Fleagle, 2013). The genus *Macaca* consists of 22 species and 37 taxa and is one of the most diverse Old World monkey genera. *Macaca* (macaques) diverged 5.1 million years ago and today is represented by an African lineage comprised of a single species *M. sylvanus*, and an Asian lineage consisting of well-defined species groups (*fascicularis, sinica, mulatta, nemestrina, Sulawesi*) inhabiting India and Asia, SE Asia and Sundaland (Ziegler et al., 2007).

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(*Trachyithecus delacouri*), and Hatin langurs (*Trachypithecus hatinhensis*) I. Positional behaviour. *American Journal of Physical Anthropology,* 128: 371-380.

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PARTICULARS OF THE CANDIDATE

NAME OF THE CANDIDATE	:	PHOEBE LALREMRUATI
DEGREE	:	DOCTOR OF PHILOSOPHY
DEPARTMENT	:	ZOOLOGY
TITLE OF THESIS	:	STUDIES OF BEHAVIOUR AND MOLECULAR CHARACTERIZATION OF NORTHERN PIGTAILED MACAQUE (*MACACA LEONINA* BLYTH, 1863) IN MIZORAM, INDIA
DATE OF ADMISSION	:	11.08.2015
APPROVAL OF RESEARCH PROPOSAL:		
1. BOS	:	15.04.2016
2. SCHOOL BOARD	:	22.04.2016
REGISTRATION NO. & DATE	:	MZU/Ph.D/927 of 22.04.2016

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Examinations	Board/University	Subject	Year	Division	Percentage
H.S.L.C.	MBSE		2007	Distinction	78%
H.S.S.L.C.	MBSE	Science	2009	II	57%
B.Sc	MZU	Zoology	2013	I	75.50%
M.Sc	MZU	Zoology	2015	I	79.50%

LIST OF PUBLICATIONS

Lalremruati, P., Zirsangzeli, Lalruatdiki & Solanki, G. S. (2017). Comparison of food preference and nutrients in captive macaques of different sexes. *Science and Technology Journal*, 5(2): 104-109.

Lalremruati, P., Vansawmkimi, & Solanki, G. S. (2017). A Comparative Study of Gestural Communication on three species of macaques (*Assamese macaque, Rhesus macaque and Pigtailed macaque*) in Mizoram. In: Sati. V.P. and Lalmalsawmzauva, K.C. (Eds.). Natural Resources Management for Sustainable Development and Rural Livelihoods (pp.1153-1164). Today & Tomorrow's Printers and Publishers, New Delhi. ISBN 8170195841.

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Patra, G., **Lalremruati, P**., Ghosh, S., Parida, A., Borthakur, S. K., & Behera, P. (2018). Prevalence of gastrointestinal parasites in captive non-human primates of zoological gardens in Northeastern region of India. *Biological Rhythm Research*. https://doi.org/10.1080/09291016.2018.1557854.

Lalremruati, P., & Solanki, G. S. (2018). Occurrence of food preference and its relation with nutritional contents. In: G. S. Solanki (ed.). Biodiversity Conservation: Strategies and Applications, page no. 207-218.

Lalremruati, P. & Solanki, G. S. (2020). Prevalence and seasonal variation of gastrointestinal parasites among captive Northern pigtailed macaque *Macaca leonina* (Mammalia: Primates: Cercopithecidae). *Journal of Threatened Taxa*, 12(3): 15370-15374.

LIST OF PAPERS PRESENTED

Occurrence of food preference and its relation with nutritional contents in captive pigtailed macaque – National Conference on Impact of Climate Change on Biodiversity: Applications of Recent Technologies for Conservation of Threatened Species, 22th to 24th September, 2016. Organized by Department of Zoology, Mizoram University.

A comparative study of Gestural Communication on three Species of Macaques in Mizoram – International Conference on Natural Resources Management for Sustainable Development and Rural Livelihoods, 26th to 28th October, 2017. Organized by Department of Geography and Resource Management, Mizoram University.

Nutrient composition and food preferences in adult males of captive stumptailed macaque – National Conference on Recent Advances in Biotechnology, 9th to 10th November, 2017. Organized by, Department of Biotechnology, Mizoram University.

Prevalence and seasonal variation of gastrointestinal parasites among captive northern pigtailed macaque – International Conference on Biodiversity, Environment and Human Health: Innovations and Emerging Trends (BEHIET 2018), 12th to 14th November, 2018. Organized at School of Life Sciences, Mizoram University.

Food preference and its relation with nutritional contents in captive nursing pigtailed macaque (*Macaca leonina*) in Mizoram – Mizoram Science Congress 2018, held at Pachhunga University College, during 4th to 5th October, 2018.

Mate choice and successful mating in captive northern pigtailed macaques – International Conference on Recent Advances in Animal Sciences (ICRAAS), 6th to 8th November, 2019, held at Pachhunga University College.

SEMINARS/TRAININGS/WORKSHOPS ATTENDED

Workshop on Northeast India Biodiversity Portal, organized by Ashoka Trust for Research in Ecology and the Environment (ATREE) in collaboration with Mizo Academy of Sciences on 7th May, 2016.

One Week Course on Research Methodology for Research Scholars held from 20 to 26th June, 2016.

International Workshop on Snakebite Management, held during 27th June to 3rd July, 2016, organized by Department of Zoology and Department of Biotechnology, Mizoram University.

Hands on Training on DNA Barcoding and Phylogenetics, held during 20 to 25th March, 2017, organized by Advanced Level State Biotech Hub Facility, Department of Biotechnology, Mizoram University.

Workshop on Statistical and Computing Methods for Life Science Data Analysis, held during 5th to 10th March, 2018, organized by the Biological Anthropology Unit, ISI, Kolkata and Department of Botany, Mizoram University.

Seminar on Science and Technology for a Sustainable Future, held at Seminar Hall, Pachhunga University College, on 30th April, 2018.

Training Programme on "Understanding the Impact of Forest Fire on the Faunal Resources of North Eastern States," organized by Zoological Survey of India, Kolkata.