SURVEY STUDY ON SOUTHERN PIGTAILED MACAQUE

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Abstract- ***The species epithet, nemestrina, is an adjective (derived from Latin Nemestrinus, the god of groves) modified to agree in gender with the feminine generic name.Macaca nemestrina formerly included the northern pig-tailed, Pagai Island, and Siberut macaques as subspecies.All four are now considered separate species. Macaca nemestrina can reach a weight of 5–15 kg in large males. These monkeys are buff- brown with a darker back and lighter lower parts of the body. Their common name refers to the short tail held semi-erect and reminiscent of the tail of a pig.***

Keywords- Macaca Nemestrina, Survey, Morphology, Habitate and distribution, Behaviour and Ecology.

I. INTRODUCTION

Pigtail macaques are widely distributed throughout Southeast Asia in the oriental biogeographic region. They are found in many countries including India (northeast), China (south), Indonesia (Borneo, Kalimantan, Sumatra), Bangladesh (east), Burma, Thailand, Laos, Cambodia, Vietnam, Malaysia (Malay Peninsula) (Cawthon Lang, 2009). Also found in Assam, Yunnan, Indochina, Bangka, and neighboring islands (Nowak, 1999). (Cawthon Lang, 2009; Nowak, 1999)Macaque species are often capable of being introduced into other areas of the world with success. Pigtail macaques have been introduced in Singapore and the Natuna Islands (Nowak, 1999). (Nowak, 1999). Pigtail macaques live in elevations starting at sea level and ranging to above 2000 m. They live in forests, mostly rainforests, and swamps. They prefer dense, humid rainforest with temperatures ranging from 18 to 30 degrees Celsius (64 to 86 Fahrenheit). Temperatures change seasonally and vary regionally. Rainforests they inhabit also get more than 2500 mm (8.20 ft) of rain each year. (Cawthon Lang, 2009). Pigtail macaques get their name from a unique feature of their morphology. Their short tails, which they carry half- erect, resemble the tails of pigs, thus giving them their name "pigtail" macaque. Their tails also have very little hair or no hair at all (Cawthon Lang, 2009). Tail length for females varies from 130 mm to 253 mm and for males the tail length varies from 160 mm to 245 mm (Rowe, 1996). (Cawthon Lang, 2009; Rowe, 1996)

Pigtail macaques have light brown hair covering their bodies and white underbellies. The hair on the top of their heads is either dark brown or black and grows so that it looks like they have an indentation on the tops of their heads (Cawthon Lang, 2009). Males have mane-like hair around their faces (Wildscreen, 2003). Pigtail macaques also have long legs and hairless snouts (Wildscreen, 2003). Infant pigtail macaques are born black and develop adult coloration as they age (Cawthon Lang, 2009). (Cawthon Lang, 2009; "ARKive: Images of Life on Earth", 2003)

Pigtail macaques are sexually dimorphic, with males being larger (Cawthon Lang, 2009). Females are roughly half the size of males (Wildscreen, 2003). The average length of males varies from 495 mm to 564 mm. The average weight of males varies from 6.2 kg to 14.5 kg. The average length of females varies from 467 mm to 564 mm. The average weight of females varies from 4.7 kg to 10.9 kg (Cawthon Lang, 2009; Rowe, 1996). Males also have large canine teeth that average 12 mm in length. These teeth are often used in agonistic encounters (Cawthon Lang, 2009). The average length of female canine teeth is 7.3 mm (Rowe, 1996). (Cawthon Lang, 2009; Rowe, 1996; "ARKive: Images of Life on Earth", 2003)The average weight of the brain of an adult pigtail macaque is 106 g (Rowe, 1996). Pigtail macaques move around on theground and throughout the trees on all fours

(quadrupedally) (Cawthon Lang, 2009). (Cawthon Lang, 2009; Rowe, 1996). Pigtail macaques are not monogamous and females will mate with multiple males during a lifetime. They do not discriminate between adolescents and adult males. When there are only a few females that are in estrus, the highest ranking males will be able to monopolize them. They can keep younger and lower-ranking males from attempting to mate and will often act aggressively toward the male and the female if the lower-ranking male attempts to copulate. However, if there are more than a few females in estrus, the top ranking males cannot effectively control females and lower-ranking males gain opportunities to copulate. When a female reaches sexual maturity at 3 years of age, she can present herself to males with her anogenital swelling during estrus for reproduction. When this time comes, the female will show her backside, including her anogenital swelling, and look over her shoulder at the male. The male will then draw back his ears and push his lips outward. (Cawthon Lang, 2009)

Although higher-ranking males are generally able to copulate more frequently with more females, this does not mean that they produce more offspring than do lower-ranking males. According to a study done with captive pigtail macaques, female rank is more important to reproductive success. It also helps to determine the sex of offspring. Higher-ranking female pigtail macaques will produce female offspring. This is because female infants are more energetically expensive. They require a lot more attention from their mothers because they stay with the group and nurse more often. Higher- ranking females can benefit from this because they gain allies in their daughters. Lower-ranking females will give birth to male offspring because they nurse less often and do not require as much attention. Once they are old enough they leave the group to join another group, hopefully gaining a higher position in that group through competition. (Cawthon Lang, 2009). Pigtail macaques are year-round breeders. However, there is a slight increase during the months of January and May. Females have reproductive cycle of about 30 to 35 days and during this time display a large, purple- pink anogenital swelling. They give birth to single infants after a gestation period between 162 and 186 days. Young pigtail macaques are then nursed for 8 to 12 months. After one year pigtail macaques are considered adolescents until they reach reproductive maturity at the age of 3 years old for females and 4.5 years old for males. (Cawthon Lang, 2009; Nowak, 1999). Females provide the majority of care for the young. Mothers nurse young, carry them, and protect them throughout their first year of life. After that they still provide some care, especially to female offspring, generally through grooming and social support. This can last throughout their whole lives or until they leave the natal group. (Cawthon Lang, 2009)

During the first month of their lives, offspring and mothers are hardly ever separated. After the fifth week though, the infant will separate from its mother and begin to explore its surroundings. This can cause problems because the infant is then in danger of being kidnapped by other adult females.
This is particularly the case when higher-ranking females seize lower-ranking female's offspring. However, if the infant is separated from its mother for too long, it will more than likely die from starvation or dehydration. (Cawthon Lang, 2009)When pigtail macaques are born they have a black coat, but by the third month of life, this starts to change to an olive brown, which is typical of adults. At one year old pigtail macaques are no longer considered infants. After one year pigtail macaques are considered adolescents until they reach reproductive maturity at the age of 3 for females and 4.5 for males. (Cawthon Lang, 2009). Pigtail macaques live in multi-male, multi-female groups. The females stay with the natal group, making it a female-bonded society. The largest group seen is 81 monkeys. The average group size is between 15 and 40 individuals. When a male is between the ages of 5 and 6, they leave the natal group and roam independently or try to join another group. If they happen to join another group, they go in as the lowest-ranking male and have to work their way up through competition with the other males. Females also have their own dominance hierarchy, with the highest-ranking females generally being sisters who share this role and are tolerant of one another. They display this by grooming, kissing, and feeding together. (Cawthon Lang, 2009; Rowe, 1996)

Males are socially dominant over females. However, groups of females will band together against a male and attack him. Sometimes females will attack lower-ranking males with the help of their relatives because of competition for food. There is also aggression between higher-ranking males and lower- ranking males. Aggression levels are especially high when solitary males are trying to join a new group. (Cawthon Lang, 2009; "ARKive: Images of Life on Earth", 2003)

After agonistic encounters, there are different forms of reconciliation, depending on gender and rank. Females may mount each other after an aggressive encounter. The dominant one will mount the subordinate one. In males it is the opposite. The dominant male will be mounted by the subordinate one, showing the dominant's tolerance of those lower than himself. Dominant females also have a way of showing their tolerance. This is generally done through the dominant female kissing the subordinate one. (Rowe, 1996)

The dominant male in a captive environment sometimes takes part in infanticide within the group. This has only been seen in captive pigtail macaques. (Cawthon Lang, 2009) Pigtail macaques are diurnal. The spend most of their time in the trees, with only 8.4% of their time on the ground. Their arboreal time is also divided between different canopy levels, with most time spent in the middle canopy (47.4%), then the lower canopy (33.8%), and finally the upper canopy (10.4%) (Rowe, 1996). Pigtail macaques cover long distances while foraging, indicating that they have large home ranges. Their home ranges vary in size from about 0.6 to 8.28 km² (0.232 and 3.20 mi²). In a day of foraging they will travel linear distances between 825 and 2964 m. Home ranges usually overlap with other groups and there has been little evidence to suggest that they defend these areas. However, when in aspecific area at a specific time, they may drive off other groups of monkeys. Larger groups might also overthrow smaller ones. (Rowe, 1996). Some researchers describe pigtail macaques as silent monkeys because they seem to be very quiet. When seen running away after an episode of crop raiding, pigtail macaques are almost completely silent. This silent tactic is not limited to simply crop raiding and shows up in most encounters where pigtail macaques are fleeing a certain area. However, they do make a lot of vocalizations. The most often used vocalization when moving through the middle and upper canopies of the rainforest is the "coo." It is generally used while pigtail macaques are foraging and can be either a short call or a long call, depending on the information being exchanged. Some other vocalizations are made when pigtail macaques are being threatened or endangered, especially during agonistic encounters with other pigtail macaques. These other sounds include "squeals," "screams," "growls," "barks," and "screeches." (Cawthon Lang, 2009)

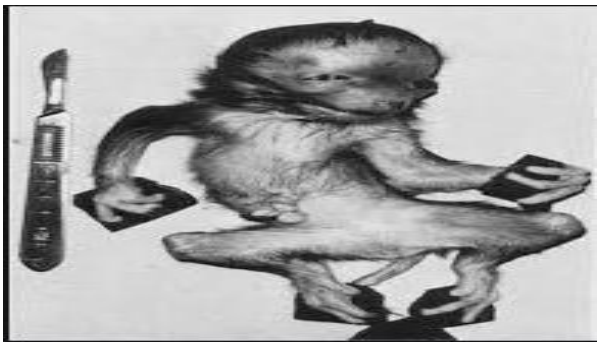
Pigtail macaques use other forms of communication like visual cues and body postures. Both males and females use a form of puckering to communicate. Males use their lips to attract females who are in estrous for mating, which generally occurs right after the communication exchange. But males also direct this facial expression to other males. In this case, it usually makes the lower-ranking male withdraw from the encounter. Another way to threaten other males is to shake branches. This is also used to attract females for copulation. Pigtail macaques use another very common facial expression that includes bared teeth and silence. However, unlike the puckering lips, lower-ranking males direct this signal to more dominant males. Females have their own form of visual cues. When in estrous they get large anogenital swellings that turn a purple-pink color. This allows males to know that they are ready for copulation. Like other primates, touch and chemical cues also are likely to play a role in social communication. (Cawthon Lang, 2009). Pigtail macaques are primarily frugivorous. The vast majority of the foods that they eat are fruits, but they also eat insects, seeds, leaves, dirt, and fungus (Cawthon Lang, 2009). Other foods in the diet of pigtail macaques include nestling birds, termite eggs and larvae, and river crabs (Rowe, 1996). Pigtail macaques are ground foragers. They divide into small groups while foraging (about 2 to 6) but keep in contact with the other groups through vocalizations. They range widely when searching for food. Pigtail macaques are known for raiding the fruit crops of farmers. They will set up a guard to look for humans and shout a warning signal to those in the fields (Cawthon Lang, 2009). (Cawthon Lang, 2009; Rowe, 1996)Research in captivity has looked at which types of fruits and vegetables are preferred by pigtail macaques. The foods chosen at the highest frequency by the pigtail macaques studied were mango and pineapple. The food chosen least was carrots (Laska, 2001). (Laska, 2001). Pigtail macaques affect their ecosystems with their foraging habits. By eating the fruits, leaves, and other vegetation they participate in spreading seeds around the forest. Their diets include many fruits, plants, fungus and other living things such as insects, nestling birds, and river crabs. (Cawthon Lang, 2009)

Pigtail macaques are also known to participate in exploitative and interference competition with white-handed gibbons (Hylobates lar). This in turn affects the amount of resources available to white-handed gibbons (Whitington, 1992). (Whitington, 1992)One study of a colony of pigtail macaques in captivity showed them to be intermediate hosts of the parasite Echinococcus granulosus. Pigtail macaques can become infected with this by eating E. granulosus eggs in the feces of canids. Canids are the definitive host of this parasite. (Plesker, et al., 2001)About 90% of macaques and old-world monkeys are infected with respiratory mites. These mites affect the lungs of the monkeys. (Kim and Kim, 2003)

A study was conducted on parasites in an outdoor breeding colony in Louisiana. The study included baboons, rhesus macaques, and pigtail macaques and the data reflect the parasites for all three species combined. The study did a fecal and blood survey of over 4000 of the animals. Endemic pathogenic intestinal parasites included Trichuris trichiura found in 35%, Strongyloides fülleborni found in 34%, Balantium coli found in 21%, and Giardia lamblia found in 0.3%. Only one endemic pathogenic blood parasite was found, which was Trypansoma cruzi in 0.8%.Pigtail macaques are classified as vulnerable on the IUCN redlist. Their vulnerability comes from many sources. The first source that poses a threat for the pigtail macaques is destruction of their natural habitat. From large scale timber companies cutting down trees to small families taking wood for fire or building, each time forests are cut, pigtail macaque habitat is destroyed. Effective protection of forested habitat and education of local people is necessary to help protect this species. (Cawthon Lang, 2009)

Pigtail macaques are often killed by locals for food. They are being shot and killed at higher rates in some places, such as Borneo, where they are becoming rare (Nowak, 1999). Pigtail macaques are also targeted in order to become the subjects of biomedical research especially for research on HIV/AIDS (Cawthon Lang, 2009). (Cawthon Lang, 2009; Nowak, 1999)

Another threat to pigtail macaques, especially in India, is the effects of the nearby coal mines. Pollution from the coal mines is harmful to the pigtail macaques that live nearby. This problem could be solved by the Indian government taking steps to regulate the coal mining system. (Cawthon Lang, 2009)Daily oral administration of 10 mg/kg retinoic acid to pregnant Macaca nemestrina monkeys on days 20 to 44 resulted in a high frequency of craniofacial and musculoskeletal malformations. Craniofacial defects including cleft palate and anomalies of the pinna were common as were ectrodactyly, kyphosis, and muscular-joint contractures. Transposition of the great vessels of the heart occurred in one animal and polycystic kidney and associated urogenital anomalies in another. Shorter treatment periods with similar or higher dosages were not teratogenic and were less fetocidal. Although only relatively long treatment courses were teratogenic, the defects that resulted were morphologically similar to those induced with retinoic acid or other vitamin A compounds in other animal orders.

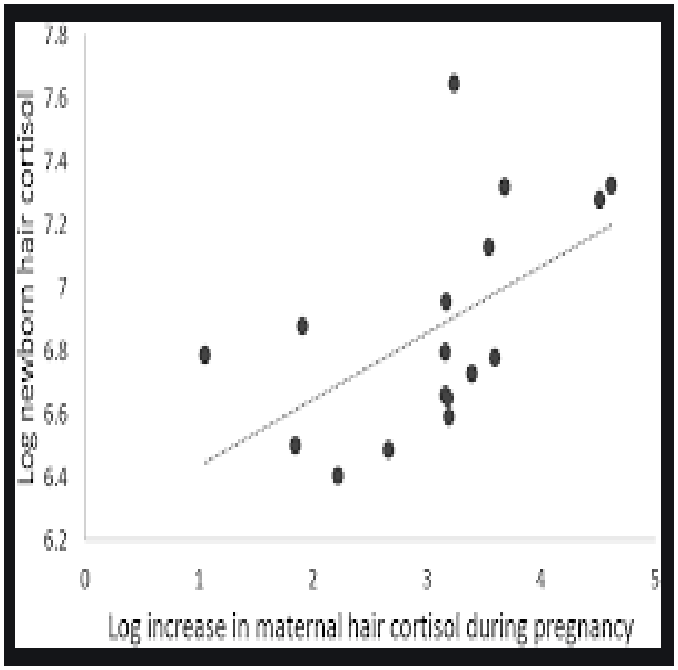


One promising conservation effort was reported in a study by Steinmetz, Chutipong, and Seuaturien (2006). They led wildlife workshops in local villages in Southeast Asia in order to teach villagers about the status of endangered animals (including pigtail macaques) and what to do to help these animals thrive. The workshops involved assessing the level of danger to the animals, determining what activities were leading to the endangerment of the species, and coming up with a plan of action to protect the species. The study also involved inter-village cooperation. Villages were brought together to understand and help these endangered animals. This study had promising results that led to less killing of pigtail macaques in the villages that participated. It is possible that implementing more educational workshops and cooperative programs could lead to helping change the vulnerable status of pigtail macaques and other species. (Steinmetz, et al., 2006). Southern pigtail macaques have olive brown fur over their entire bodies, except for their undersides, which are white. The fur on the top of their heads is dark brown or black and grows in a pattern that makes them look like there is a depression in the center of the top their heads (Rowe 1996; Groves 2001). Northern pigtail macaques have golden brown fur and the fur on the top of their heads is brown. They have red streaks of fur extending from the outer cornier of each eye towards the ear. Pigtail macaque infants are born black and as they mature, their pelage changes to the adult coloration (Crockett & Wilson 1980). Pigtail macaques are sexually dimorphic with males measuring 495 to 564 mm (1.62 to 1.85 ft) and weighing 6.2 to 14.5 kg (13.7 to 32.0 lb) while females measure 467 to 564 mm (1.53 to 1.85 ft) and weigh between 4.7 and 10.9 kg (10.4 and 24.0 lb) (Fa 1989). Males have much larger canine teeth than females, measuring 12 mm (.472 in), on average, which are used in aggressive interactions (Rowe 1996). Pigtail macaques have an abbreviated tail, less than the length of the body from head to rump, which is often bare or covered only by sparse fur (Rowe

1996; Groves 2001). Pigtail macaques get their popular name from their tails, which are short and carried half-erect so that they somewhat resemble a pig's tail (Choudhury 2003). They move quadrupedally on the ground and through trees (Rowe 1996). Pigtail macaques have an average lifespan of about 26 years (Sponsel et al. 2002). Pigtail macaques have a wide range throughout Southeast Asia. Southern pigtail macaques are found in northeastern India, southern China, in Indonesia on Borneo (Kalimantan) and Sumatra, in eastern Bangladesh, as well as in Burma, Thailand, Laos, Cambodia, and Vietnam. They are also found in Malaysia, both on the Malay Peninsula and on Borneo (Feeroz et al. 1994; Groves 2001; Choudhury 2003). Northern pigtail macaques are found in peninsular Thailand , through Burma and Indochina and into Bangladesh , India , and Southern China . There is some evidence of hybridization of the two species in Thailand, but interbreeding is not widespread (Groves 2001).Pigtail macaques are found in lowland and hilly primary rainforests and occasionally are found in swamp and secondary forests (Crockett & Wilson 1980). They prefer undisturbed forests and are found in the highest densities in intact rainforests (Supriatna et al. 1996). Rainforests are maintained in warm and humid climates where temperatures range between 18 and 30° C (64 and 86° F) and where there is more than 2500 mm (8.20 ft) of rainfall each year, though there may be seasonality in rainfall. Where they are found in Bangladesh, the annual precipitation is 2034 mm (6.67 ft) with the highest rainfall occurring during the monsoon season, from May to September, and the lowest in December. The coldest months of the year are December and January, which have average temperatures of 12.3° C (54.4° F) and 9.7° C (49.5° F), respectively. August is the warmest month of the year with an average maximum temperature of 33° C (91.4° F) (Feeroz et al. 1994). In neighboring northeastern India, the climate and rainfall is quite variable, ranging from less than 1000 mm (3.28 ft) to more than 10,000 mm (32.81 ft) annually (Choudhury 2003). The climate in this part of India can also be as cool as 4°C (39.2° F) in December to early February to 30° C (86° F) from June to August. Most of the rain occurs from May to September, and snow falls in winter at the higher altitudes (Choudhury 2003). Rainfall in southern Sumatra ranges from 2000 to 3267 mm (6.56 to 10.7 ft) per year with indistinct wet or dry seasons (Supriatna et al. 1996). The climate there is also warm and humid (Lucas & Corlett 1991). In Peninsular Malaysia, rainfall averages 2100 mm (6.89 ft) per year with the least amount of rainfall during January and February and the period of highest rainfall occurring during September and October (Saiful & Nordin 2001).Pigtail macaques range from sea level to above 2000 m (6562 ft) (Srivastava & Mohnot 2001; Choudhury 2003). Pigtail macaques are 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, dirt, and fungus (Crockett & Wilson 1980; Caldecott 1986). Pigtail macaques spend most of their time on the ground, but the northern pigtail macaque seems to be more arboreal than the southern species (Maestripieri pers. comm.). Spending most of their time on the ground foraging, they are particularly adept 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. Pigtail macaques are especially likely to raid crops during rainstorms, when farmers are inside, away from their crops (Crockett & Wilson 1980). In some areas of the Malay Peninsula, farmers keep and train pigtail macaques to retrieve coconuts and fruits from cultivated trees (Crockett & Wilson 1980; Sponsel et al. 2002). To understand non- human primates and to provide them with good welfare it is important to know how they perceive the world and communicate among themselves. Of all the animals used in the laboratory, the perceptual world of the non-human primates is assumed to be most similar to that of man, in particular because of our shared refined visual capabilities. However, there are important differences between the sensory capabilities of non-human primates when compared with man, and there are genera and some species differences too. This article summaries the sensory capabilities of the non-human primates commonly used in the laboratory, highlights important modes of communication, and identifies several implications of these for designing and refining experiments, housing and husbandry systems and enrichment strategies. The sexual behavior of 4 adult male pigtailmacaques was recorded during two tests with each of 2 estrogen-primed females. The behavior of the males was highly consistent across tests and females but, quantitatively, varied significantly from male to male. The pigtails were multiple mount ejaculators that achieved one to three ejaculations in 1 h. A median number of eight intromissive mounts, separated by intermount intervals of 1.6 min, preceded first ejaculations. Median ejaculatory latency was 18.5 min, while the postejaculatory interval was 33 min.Each male had a characteristic number of thrusts and rate of thrusting per mount. The group medians were 12 thrusts per mount and 2.3 thrusts per sec, respectively. The multiple mount pattern of the male pigtails resembles that of the rhesus and Japanese macaques but differs from the single mount pattern of the bonnet and stumptail macaques.

Cortisol is a well-known glucocorticoid that can be used as a biomarker of hypothalamic-pituitary-adrenocortical activity. To explore basal cortisol physiology during pregnancy and infancy in Macaca nemestrina monkeys, hair was collected from a convenience sample of 22 healthy mother–infant dyads. Adult females were housed in pairs as

part of a small breeding colony at the Washington National Primate Research Center and infants were reared in a specialized nursery. Maternal samples were collected from females during a pregnancy-detection ultrasound and immediately following labor and delivery. Infant samples were collected at birth, 20 days, 4, 6, 8, and 10 months of age. Hair cortisol concentrations (HCCs) were determined using an enzyme immunoassay in washed and ground hair samples. Like human mothers, macaque HCCs rose during pregnancy (paired $t = 5.8$, $df = 16$, $P < 0.001$). Maternal HCCs at pregnancy-detection (114.2 ± 12.07 picogram/milligram [pg/mg]) were highly predictive of maternal HCCs at delivery (144.8 ± 13.60 pg/mg), suggesting a trait-like quality ($r = 0.90$, $P < 0.001$). When maternal HCCs were viewed on a continuum, the absolute rise in cortisol over the course of pregnancy was significantly related to newborn HCCs ($r = 0.55$, $P = 0.02$). Infant birth HCCs ($1{,}027.43 \pm 97.95$ pg/mg) were seven times higher than maternal HCCs at delivery (paired $t = 19.1$, $df = 16$, $P < 0.001$). Higher birth HCCs were strongly associated with larger decreases in infant hair cortisol until 6 months of postnatal age when infant HCCs converged on values indistinguishable from adults. Overall, study results demonstrate a marked degree of fetal cortisol exposure during the latter part of gestation and suggest that the rise in maternal cortisol over pregnancy may play an influential role on HCCs in the newborn.



Because of their large group size, between nine and 81 individuals and larger, pigtail macaques often split up into foraging groups to decrease direct competition for fruit at feeding sites. They travel in small subgroups, from two to six monkeys, along the ground, foraging as they move and keeping in contact with other subgroups through vocalizations (Crockett & Wilson 1980; Caldecott 1986). In addition to spreading out over the landscape as they forage, pigtail macaques cover large areas each day. They have home ranges between .6 and 8.28 km² (.232 and 3.20 mi²) and in areas of high density, groups' home ranges can overlap each other by as much as 50% (Sponsel et al. 2002). The day range length varies between 825 and 2964 m (.513 and 1.84 mi), depending on weather conditions and seasonal fruit availability (Caldecott 1986). Pigtail macaques are notable for their arched, intermediate-size tails that are bare, or nearly bare, at the end (i.e. pig-like), from which their common name derives. Southern pigtail macaques (M. nemestrina) have olive brown fur with white fur on their undersides. The fur of northern pigtail macaques (M. leonina) is golden brown with streaks of red fur extending from the outer edges of the eyes to the ears. Behavioral studies of free-ranging pigtail macaques have only been conducted on southern pigtail macaques and the taxonomic status of pigtail macaques in captivity whose behavior has been studied is problematical (Groves, 2001).

Five monkey brainstems (three Macaca nemestrina, obtained from Washington National Primate Research Center, two Macaca mulatta sections from previous studies—all fixed with 4% paraformaldehyde), and four post mortem human cases (fixed in 10% formaline) with no prior oculomotor symptoms obtained from the Reference Center for Neurodegenerative Disorders of the LMU were examined. Free-floating monkey brainstem sections were processed for the simultaneous immunofluorescence detection of one K+ channel together with either SMI-32, a non-phosphorylated neurofilament (NP-NF) marker, or perineuronal net (PNN) marker hyaluronan and proteoglycan link protein 1 (HAPLN1), or with γ-aminobutyric acid (GABA)-A receptor (GABAAR). Sections were subsequently visualized with a laser-scanning confocal microscope (Leica SP5, Mannheim, Germany) as described previously (May et al., 2016). Paraffin sections from human and monkey brainstems were processed for the detection of one K+ channel together with either SMI-32 or PNN marker aggrecan (ACAN) using an immunoperoxidase protocol (see Table 1). The specificity of antibodies was validated by antibody-antigen preabsorption tests (data not shown). Since K+ channels have been extensively studied in the auditory nuclei, the medial superior olive (MSO) in the same sections served as the internal positive control for both species (Johnston et al., 2010; Mathews et al., 2010).

Macaque monkeys (Macaca nemestrina) are typically used in our laboratory's optical imaging studies. A 25-mm 'optical window' for acquiring optical and electrophysiological recordings is constructed over the hand motor cortex. This procedure involves a craniotomy and mounting a specially designed stainless steal chamber over the cortex. We typically choose hand motor cortex in optical imaging studies of epileptiform activity; in this way, electrophysiological activity from the cortex can be acquired simultaneously with observations of hand motor movements and EMG recordings from hand muscles. The cortex is stimulated with a bipolar stimulating electrode (5 mm inter-electrode distance) powered by an Ojemann Cortical Stimulator (Integra Life Science Corporation). In the studies shown here, the cortex was illuminated with either 535- or 660-nm light. Images are acquired with at 16-bit cooled digital CCD camera (Roper Scientific) mounted on an operating microscope.

A typical data acquisition trial involves acquiring a series of images at a rate of at least 5 Hz continuously for several minutes. Each trial consists of a 20-s control period prior to stimulation, followed by 4 s of electrical stimulation of the cortex, and ending with a 2–3 min recovery period. Percent- difference-images are generated by subtracting and then dividing the first control-image from all of the other images acquired during the same data-acquisition trial. The percent- difference-images are pseudocolored to facilitate the visualization of small optical changes. Primary osteoarthritis has been reported in cynomolgus, rhesus and pigtailed macaques, baboons, and owl monkeys (Carlson et al., 1994; Chateauvert et al., 1989; Rothschild, 1993; Rothschild et al., 1999; Rothschild and Woods, 1992). This degenerative joint disease is characterized primarily by progressive destruction of articular cartilage matrix, bone abnormalities and minor synovial changes. It increases in prevalence and severity with advancing age and can affect the spine, knee, hip, elbow, wrist and interphalangeal joints. Female rhesus macaques showed a higher frequency of disease than did males in one study (DeRousseau, 1988). There was no correlation between articular lesions of the knee and factors of gender and weight in a study of cynomolgus macaques (Carlson et al., 1996). Increased parity in rhesus macaque females was associated with increased frequency in another study (Chateauvert et al., 1989). Caging did not affect the incidence of osteoarthritis; however, caged monkeys had less severe disease than did their free ranging counterparts (Chateauvert et al., 1989). Clinical signs may be absent or include decreased range of motion, decreased activity, gait abnormalities, crepitus, and joint enlargement and deformity. Radiographs often reveal narrowing of the joint space, increased thickness of the subchondral bone, subchondral bone cysts and osteophyte formation at the joint periphery. Gross softening, fibrillation, erosion, and ulceration of the articular cartilage may be observed as well as eburnation of subchondral bone and osteophytosis. Key microscopic features include fibrillation, clefting and erosion of the cartilage matrix, chondrocyte necrosis, proliferation of chondrocytes, and subchondral bone proliferation (Carlson et al., 1994; Chateauvert et al., 1989). Hyperplasia of the synovial lining cells and a minimal inflammatory infiltrate composed of few lymphocytes and plasma cells may be present (Carlson et al., 1994).

The pathogenesis of this disorder is unknown and it is not clear if the initiating changes occur primarily in the articular cartilage or in the subchondral bone (Bailey and Mansell, 1997). Both structures are important for synovial joint health and function and defects in either are potential causes of lesions in the other. Plasmodium knowlesi is a malaria parasite that is found in nature in long-tailed and pig-tailed macaques. Naturally acquired human infections were thought to be extremely rare until a large focus of human infections was reported in 2004 in Sarawak, Malaysian Borneo. Human infections have since been described throughout South-East Asia, and P. knowlesi is now recognized as the fifth species of Plasmodium causing malaria in humans. The molecular, entomological, and epidemiological data indicate that human infections with P. knowlesi are not newly emergent and that knowlesi malaria is primarily a zoonosis. Human infections were undiagnosed until molecular detection methods that could distinguish P. knowlesi from the morphologically similar human malaria parasite P. malariae became available.

P. knowlesi infections cause a spectrum of diseases and are potentially fatal, but if detected early enough, infections in humans are readily treatable. In this review on knowlesi malaria, we describe the early studies on P. knowlesi and focus on the epidemiology, diagnosis, clinical aspects, and treatment of knowlesi malaria. Effective colony management is critical to guarantee the availability of captive NHP as subjects for biomedical research. Pigtailed macaques (Macaca nemestrina) are an important model for the study of human and nonhuman primate diseases and behavior. Johns Hopkins University hosts one of the largest captive colonies of pigtailed macaques in the United States. In this study, we used 56 single-nucleotide polymorphisms (SNP) to characterize this population of pigtailed macaques, understand their population structure, and assess the effectiveness of their colony management. The results demonstrate that the colony has maintained a high level of genetic diversity, with no loss of heterozygosity since its origin, and low levels of inbreeding and genetic subdivision.

II.LITERATURE REVIEW

NHP are widely bred in colonies in the United States as models for biomedical and biobehavioral research. In addition to the more commonly used macaque species— rhesus macaques (Macaca mulatta) and cynomolgus macaques (also called long-tailed macaques or crab-eating macaques, M. fascicularis)—pigtailed macaques (M. nemestrina) have been widely used as models for studies of infectious diseases (including HIV infection and AIDS) immunology, neuroscience, pathology, and behavior. Because of their outgroup evolutionary relationship with rhesus and cynomolgus macaques, pigtailed macaques also serve as a valuable comparison group in comparative evolutionary studies, such as those focusing on MHC compatibility and its relationship to infectious disease susceptibility.Wild pigtailed macaques are widely distributed— from northeastern India, Bangladesh, southern China, and Vietnam to Peninsular Malaysia, Borneo, and Sumatra.Although once considered to be the same species (M. nemestrina), southern pigtailed macaques (M. nemestrina, the assumed species of the pigtailed macaques that are currently captively bred in the United States) and northern pigtailed macaques (M. leonine) were later classified as separate species based on morphology8 and mitochondrial DNA evidence. The overlap between the geographic distributions of these 2 species in the wild with those of other macaque species, such as cynomolgus macaques, suggests that admixture between M. nemestrina and M. leonina or other species is possible. However, no evidence has yet confirmed admixture, and small amounts of admixture in the early phylogenetic history of each species could result in little observable phenotypic difference.Captive colonies are often derived from a small number of founders that, together with the typically high variance in male reproductive success and low sex ratio in most captive macaque colonies, could lead to a small effective population size. Genetic heterogeneity is lost at a rate inversely proportional to the effective population size, with a resultant loss of fitness over subsequent generations due to inbreeding depression and genetic subdivision. Consequently, such colonies should be genetically monitored so that genetic management procedures can be implemented to minimize loss of colony fitness and maximize genetic heterogeneity.In addition to its use as a tool for colony management, population genetic analysis of colonies—such as estimates of the degree and distribution of genetic heterogeneity, coefficients of kinship and inbreeding, admixture, and identification of the ancestral origin of the founders of the colony—is valuable in assessing the suitability of animals as models for the study of particular diseases.

A previous study used 18 short tandem repeat markers (STR) to genotype the captive pigtailed macaque colony at the Washington National Primate Research Center.The results revealed that the Washington colony of pigtailed macaques exhibits greater genetic heterogeneity than most captive colonies of rhesus macaques. Through careful genetic management, the colony has successfully maintained maximum founder representation and low levels of inbreeding.A second colony of 158 pigtailed macaques, whose genetic structure has not been reported previously, has been maintained at Johns Hopkins University since 1999. This colony was established by the introduction of approximately 100 pigtailed macaques acquired from the following breeding facilities throughout the United States during the year indicated in parentheses: the Tulane National Primate Research Center (1999), Covance (2003), Laboratory Animal Breeders and Services of Virginia (2003), Pennsylvania State University–Hershey (2005), the Yerkes National Primate Research Center (2006), Alpha Genesis (formerly known as Laboratory Animal Breeders and Services of Virginia, 2007), the Washington National Primate Research Center (2008), and the New Iberia Primate Research Center (2009). The initial colony was established using pigtailed macaques obtained from 3 of the 7 listed breeding facilities (Tulane, Covance, Laboratory Animal Breeders and Services of Virginia), which produced the first offspring cohort, born between 2003 and 2008. However, founding yearlings from the 4 other breeding facilities (Pennsylvania State University–Hershey, Yerkes National Primate Research Center, Washington National Primate Research Center, and New Iberia Primate Research Center) were introduced to the colony between 2005 and 2009. Yearlings began breeding by 2008, when pairings of sires and dams were organized to randomize breeding and avoid inbreeding. Between 2003 and 2012, the founding colony experienced 200 new births, from which all surviving females and a few males were retained as future breeders.Surviving macaques from the founder group that gave birth to the first cohort of offspring (born in 2003) included approximately 6 males and 21 females. During subsequent years, the number of breeders varied from 8 to 13 males and 33 to 70 females, with an average effective population size of approximately 32 animals (an estimate based on the assumption that males and females become sexually mature at 4.5 and 3.5 y of age, respectively).

Given the heterogeneous origins of the colony, we expected a relatively high level of genetic heterogeneity, as well as a significant level of population structure due to strong genetic subdivision (although this genetic subdivision can be mitigated by careful selective breeding). Moreover, because

macaques of different ages were acquired from different breeding facilities and introduced at different times during the development of the colony, significant changes in the genetic structure of the initial offspring cohorts might have occurred during the early stages of the colony's development.In addition to short tandem repeats, single-nucleotide polymorphisms (SNP) have been used in population genetics with increasing frequency. In mammalian genomes, SNP are more abundant than STR, showing closer linkage to sites of interest, and are easily adaptable to high-throughput analysis with high accuracy in genotype calls.Due to advances in DNA sequencing technologies, the development and analysis of SNP markers are now less costly than those of STR and can be applied to both captive and wild animals of all primate species.In the current study, we used SNP to genotype the pigtailed macaques at Johns Hopkins University to characterize the genetic structure and heterogeneity of the founders and subsequent birth cohorts of this captive colony. In humans, about 20 % of all primary intracranial tumors are meningiomas (Louis et al., 2000) and about 80–90 % of these are regarded as benign lesions (Whittle et al., 2004; Goldstein and Harsh, 2005; Harter et al., 2017). Meningioma most commonly develops in older people, and women are more often affected than men (Fonkem et al., 2016; Kalamarides and Goutagny, 2006; Louis et al., 2000; Longstreth Jr. et al., 1993; Wiemels et al., 2010; Whittle et al., 2004; Perry, 2006). In humans, the tumor is generally tightly attached to the dura mater (Nagashima et al., 2006). It often shows a slow expansive growth with compression of adjacent brain tissue and grows along the extensions of the dura mater (Whittle et al., 2004). The tumors are often well demarcated but might also show infiltrative growth (Perry et al., 1999).

Meningiomas are relatively common in cats (Zaki and Hurvitz, 1976) and dogs (Zaki and Hurvitz, 1976). In cats, 59 % of all intracranial tumors are meningiomas (Troxel et al., 2003) and 45 % of all intracranial tumors are meningiomas in dogs (Snyder et al., 2006). However, meningiomas are rare in cattle, sheep, and horses (Cantile and Youssef, 2016; Koestner and Higgins, 2002; Summers et al., 1995). Meningiomas are also known to occur in laboratory animals such as rats (Mitsumori et al., 1987) and mice (Summers et al., 1995).

Few studies have reported findings of meningiomas in nonhuman primates (Lowenstine, 1986; McClure, 1980). In prosimians, Winkelmann et al. (2007) reported a psammomatous meningioma in a black-and-white-ruffed lemur (Varecia variegata variegata), and Remick et al. (2009) documented a case of an anaplastic meningioma in a collared brown lemur (Eulemur collaris). In monkeys, Jungherr (1963) summarized necropsy results of 12 000 cynomolgus/rhesus monkeys, and observed one case of meningiomatosis in the lumbar cord. McConnell et al. (1974) briefly mentioned a meningioma in a survey of free- living chacma baboons in South Africa (Papio ursinus). In 2011, Oliveira et al. described an intracranial meningioma in a baboon (Papio spp.). Tanaka and Canfield (2012) published a case report of an intracranial meningioma with ophthalmoplegia in a rhesus macaque (Macaca mulatta).

Since reports of meningiomas in nonhuman primates are rare in the literature, we describe a case of a spontaneous meningioma in an aged pig-tailed macaque in this report. Histologically, the tumor displayed features of both meningothelial meningioma and of microcystic meningioma. The affected animal was a female pig-tailed macaque (Macaca nemestrina) of at least 24 years of age. The exact date of birth was not documented. The pig-tailed macaque was obtained from a breeding colony in Slovenia and arrived at the German Primate Center in Göttingen, Germany, in 1993. In 1995, it was transferred to the Paul- Ehrlich-Institut (PEI) in Langen, Germany, where it lived for 21 years in an experimental indoor facility. It was group- or pair-housed in accordance with European and German animal welfare legislation and produced seven offspring. The monkey was used for experimental blood collection. The cage was made of steel with a size of 300 cm × 375 cm × 225 cm. Large windows allowed the monkey to watch the outside environment. Natural branches, ropes, nets, bedding, mirrors, kong toys, puzzle feeders, prima-hedrons, music, and television were supplied for environmental enrichment. The diet consisted of monkey pellets ad libitum (Trio Munch®, Special Diet Services/Mazuri, Witham, England) in the morning and seasonal vegetables and fruits twice weekly. The monkey was also offered a mixture of nuts, mealworms, rice, popcorn, and curd. The monkey had acquired multiple bite injuries on different parts of its body during its time in the PEI group housing facility. It additionally had slow, insecure, and weak movements, and its vision had deteriorated progressively over the past 3 years. The toes of the left foot had been kept in a rigid claw- like grasping position for at least 5 years. Over the past 3 years, the animal developed two slowly growing subcutaneous tumors with a size of 4×3 cm each at the ventral abdomen close to the linea alba. A general atrophy of both the epaxial and the appendicular muscles became obvious during the last year before its death.

The proximal cause for the euthanasia of the animal was a combination of a laceration of the skin and muscle on the left arm and pain vocalization during walking and climbing movements within the cage.The animal was euthanized by intravenous injection of T 61 (Intervet Deutschland GmbH, Unterschleißheim,

Germany) under deep ketamine–xylazine anesthesia (Ketamin 10 %, WDT, Garbsen, Germany; Rompun®, Bayer Vital GmbH, Leverkusen).

Necropsy was performed immediately after euthanasia. Photographs were taken and organs of interest were fixed in 4 % formaldehyde solution for 7 days before processing. Paraffin embedding of fixed tissues, preparation of 4 µm sections, and hematoxylin–eosin staining were carried out in accordance with standard procedures (Mulish and Welsch, 2015). Bones were decalcified with 5–15 % hydrogen chloride (Decal®, SERVA Electrophoresis GmbH, Heidelberg, Germany) for the production of histological slides according to manufacturer's instructions. Immunohistochemical examinations were performed on paraffin-embedded sections using the following primary antibodies commercially available from DakoCytomation GmbH, Hamburg, Germany: anti-Ki67 antibody (monoclonal mouse anti-human Ki67 antigen, clone MIB-1, 1:50), anti-vimentin antibody (monoclonal mouse anti-human vimentin antigen, clone V9, 1:100), anti-cytokeratin antibody (monoclonal mouse anti-human multi-cytokeratin, clone MNF116, 1:100), anti-GFAP antibody (polyclonal rabbit anti-human glial fibrillary acidic protein, 1:500), anti-S100 antibody (polyclonal rabbit anti-human S100A1, 1:1000), anti-NSE antibody (monoclonal mouse anti-human neuron specific enolase, clone BBS/NC/VI-H14, 1:400), and anti-SMA antibody (monoclonal mouse anti-human smooth muscle actin, clone 1A4, 1:400). Immunohistochemistry was performed in an automated immunostaining system (Discovery XT, Roche Diagnostics GmbH, Mannheim, Germany) using the SABC (streptavidin–biotin complex) method and DAB (diaminobenzidine tetrahydrochloride) for signal detection (DAB Map Kit, Roche Diagnostics GmbH, Mannheim, Germany). All primary antibodies used in this case report have previously been validated and successfully used in rhesus macaques, a closely related macaque species (Gruber-Dujardin et al., 2017; Vogel and Fritz, 2003). Corresponding tissue sections from rhesus macaques were used as positive controls to demonstrate antibody specificity. Pure antibody diluent instead of primary antibody was applied to the negative control sections to visualize possible nonspecific binding of the secondary antibody. Immunohistochemical staining for epithelial membrane antigen (EMA) was performed using the EnVision Detection System (Agilent/Dako, Denmark) and the commercially available monoclonal antibody EMA (Clone E29, Ready-to-Use) was employed. Samples were visualized with the EnVision FLEX System (Autostainer Link 48, Agilent/Dako, Denmark). At necropsy, a tumorous mass was detected at the base of the ossified cranium after the brain was removed. It was centered around the hypophyseal stalk, extending cranially toward the optic chiasm, and a thin tumor tissue layer extended caudally towards the foramen magnum . The tumor was well vascularized and primarily light red or beige in color, although some areas had light grey elements. It had an elastic consistency, and some regions were slightly edematous. Its surface was mainly smooth, but revealed a slightly rough surface in the thinner caudal parts of the tumor. The tumor was firmly attached to the dura mater, well demarcated, and did not invade the brain macroscopically. The spinal cord was not examined. The paranasal sinuses showed no abnormalities. In the skull cross section, the hypophyseal fossa was completely filled with tumor tissue, which was demarcated by a red margin from surrounding bones. The cross section revealed two additional firm white parts of the tumor (2 cm × 1.5 cm and 3 cm × 1.5 cm) located in the median between the epithelium of the pharynx and parts of the sphenoidal and occipital bones, compressing both the pharyngeal and the esophageal lumen. The two oval tumors on the ventral abdominal wall were identified as lipomas. Several joints displayed arthrosis of the cartilage. In addition, spondylosis was detected in the thoracic and lumbar portion of the spinal column. A slight scoliosis was also present in the thoracic area. In the right ovary, a 0.5 cm diameter large thin-walled cyst was evident containing clear watery fluid.

The intracranial tumor completely filled the space around the pituitary gland (fossa hypophysealis). However, there was no infiltration into the pituitary gland. In contrast, there was extensive invasion into surrounding bones. Tumor cells were also attached to the perineurium of the optic nerve at the connection to the eye.

The tumor displayed two main histological cell types. In subepithelial areas of the pharynx and around the pituitary gland, the tumor consisted of multiple ovoid islands or nests of tumor cells separated by fine junctions of fibrous tissue. Occasionally, cells were arranged in indistinct whorls. The islands consisted of numerous small polygonal cells with indistinct cell borders and with moderate amounts of eosinophilic cytoplasm. Nuclei were uniform, round to ovoid, and condensed with finely stippled nuclear chromatin. Only one nucleolus was normally visible. There was mild anisokaryosis and anisocytosis, and mitotic figures were rarely observed. Overall, an island-like hepatoid appearance of the tumor with partly whorl-like layers of cells was the prominent histological characteristic consistent with human World Health Organization (WHO) grade I meningothelial meningioma. Within these areas, the tumor produced few small spots of dystrophic lamellar calcification (psammoma bodies) and very few areas with regional mucin production. This histological appearance occurred in about 65 % of the tumor mass. Tumor cells in surrounding bones had larger amounts of vacuolated, apparently empty pale cytoplasm and

smaller, more condensed nuclei.These cells also exhibited mild anisokaryosis and anisocytosis, while mitoses were infrequent. The separating fibrous tissue was also vacuolated. This histological appearance is consistent with human WHO grade I microcystic meningioma and was evident in approximately 35 % of the tumor mass.

The tumor showed immunoreactivity for vimentin (100 % of tumor cells) and very few tumor cells stained positive for Ki67. However, the tumor was negative for cytokeratin, S 100, glial fibrillary acidic protein (GFAP), neuron-specific enolase (NSE), smooth muscle actin (SMA), and EMA, while positive controls demonstrated the specificity of the antibodies. Clinical signs of meningiomas are normally the result of the compression of neighboring structures, and are therefore dependent upon tumor location (Perry, 2006; Summers et al., 1995; Whittle et al., 2004). Common clinical signs in dogs and cats are altered consciousness, seizures, and vestibular dysfunction (Motta et al., 1987). In this case, the monkey had a history of poor vision and slow, insecure, and weak movements. While these manifestations could have different causes (for example, joint alterations as the cause of slow movements), it cannot be excluded that they may be caused by the meningioma. In this context, visual impairment was reported as one clinical sign in a baboon with meningioma (Oliveira et al., 2011). In addition, eye muscles were affected in a rhesus macaque with a meningioma (Tanaka and Canfield, 2012). It is noteworthy that in our case no histological changes were detected within the eyes. However, parts of the meningioma in our case were evident close to the optic nerve, a condition that is reported in domestic animals as well (Koestner et al., 1999), which can also cause visual impairment in humans (Li et al., 2017). While further histological investigation of the nerve was not conducted, an influence of the tumor on the vision cannot be completely excluded. Meningiomas originate from the arachnoid (Kepes, 1986) or meningeal progenitor cells (Kalamarides et al., 2011) and are normally firmly attached to the meninges. They can occur anywhere along the meninges, including the optic nerve and spinal cord. Meningiomas in humans are commonly reported in the skull vault, the skull base, sites of dural reflections, and less commonly in the optic nerve sheath and the choroid plexus. Approximately 10 % of human meningiomas arise in the spine (Whittle et al., 2004). In dogs, the olfactory bulb, frontal lobes, the floor of the cranial cavity, the optic chiasm, and the suprasellar or parasellar regions are commonly affected (Patnaik et al., 1986; Snyder et al., 2006; Sturges et al., 2008). In cats, the tela choroidea of the third ventricle and dorsal and lateral convexities are involved (Koestner et al., 1999; Troxel et al., 2003). In the present case of the pig-tailed macaque, the tumor was found at the base of the ossified cranium as previously described for a baboon (Oliveira et al., 2011).Meningiomas are typically considered to be benign tumors and are normally well-demarcated masses of soft to firm consistency (Summers et al., 1995; Whittle et al., 2004). Meningiomas normally do not invade the brain but they compress neighboring structures (Frankhauser et al., 1974). However, in this case, the meningioma showed infiltrative growth into surrounding bones, which sometimes occurs in humans (Scott, 1992; Spille et al., 2016).Canine and human meningiomas are currently histologically classified according to human WHO criteria (Louis et al., 2016), but this grading system is actually not applicable to feline meningiomas (Mandara et al., 2010). WHO classification categorizes meningiomas into 15 variants: grade I (benign, nine variants), grade II (intermediate, three variants), and grade III (malignant, three variants) according to their morphological and biological behavior. Whereas human meningiomas are histologically classified as, for example, 94.2 % for grade I, 4.2 % for grade II, and 1.57 % for grade III (Dolecek et al., 2015, for the US in 2004–2011), canine meningiomas are histologically classified as 56 % for grade I, 43 % for grade II, and 1 % for grade III (Sturges et al., 2008). In contrast, grade III meningiomas were not detected in cats (Mandara et al., 2010). Generally, metastases of meningiomas are rare in humans (Enam et al., 2005), dogs (Motta et al., 1987; Pérez et al., 2005), and cats (Dahme, 1957; Motta et al., 1987). Histologically, most meningiomas do not exhibit cellular criteria of malignancy. In our case, the cells were well differentiated and displayed only a few mitoses, which is largely consistent with benign human meningiomas (0.08±0.05 mitoses per 10 HPF for benign, 4.75±0.91 mitoses per HPF for atypical, and 19.00±4.07 mitoses per HPF for malignant) (Hsu et al., 1994).

Some authors discuss advantages of the human system of classification compared with the current WHO classification for animals (Koestner et al., 1999; Mandara et al., 2010; Sturges et al., 2008). Due to these considerations and due to the evolutionary relatedness between nonhuman primates and humans, we referred to the human WHO classification in order to classify the tumor in this case. According to this classification, we diagnosed a meningioma that showed histological appearance both of a meningothelial meningioma (65 % of the tumor mass) and a microcystic meningioma (35 % of the tumor mass).Meningiomas do not have definitive cytologic markers, and the pathologic diagnosis is usually made on the basis of tumor cytoarchitecture (Louis et al., 2016). In humans, EMA and vimentin are usually the most reliable

immunohistochemical markers (Pérez-Guiones Bacete et al., 1992; Schnitt and Vogel, 1986; Schwechheimer et al., 1984; Winek et al., 1989), although many tumors are also positive for cytokeratin (Pérez-Guiones Bacete et al., 1992; Perry, 2006; Winek et al., 1989). S-100 protein immunostaining is variable (Pérez-Guiones Bacete et al., 1992; Schnitt and Vogel, 1986; Winek et al., 1989) and GFAP expression is rare (Wanschitz et al., 1995). In humans and domestic carnivores, the MIB-1 antibody against the Ki67 antigen was successfully correlated with the histological grade of meningeal neoplastic cells (Devaprasath and Chack; 2003; Maes et al., 2005; Mandara et al., 2002). In this case, the tumor only showed immunoreactivity for vimentin (100 % of tumor cells) and a few tumor cells were positive for Ki67. However, it was negative for cytokeratin, S 100, GFAP, NSE, SMA, and EMA, which is consistent with what has been reported in other studies. However, it cannot be excluded that some of the negative staining results are false negative in the present case, which might be attributed to antigen impairment during the decalcification process prior to embedding. Lack of antibody cross reactivity with pig-tailed macaque tissue seems unlikely in view of the positive reaction with rhesus macaque tissue. However, the significance of the immunohistochemical results remains questionable in the present case and the diagnosis of meningioma mainly relies on the histological appearance of the tumor. Until now, mother-infant relationships have not been studied in a wild population of the Southern Pig- tailed Macaques Macaca nemestrina. We observed six mother-infant dyads from April 2016 to September 2016 in the Segari Melintang Forest Reserve, Peninsular Malaysia using focal sampling methods from the perspectives of both individuals. We hypothesized that as infant age increased, the same important mother-infant behaviours, previously observed to change in captive pig- tailed macaque mother-infant studies, would also change over time in field conditions. We expected that as the infant ages, mothers would decrease their rates of restraint and retrieval, and increase their rates of punishment. Two separate generalized linear mixed models (GLMM) of mother permissive behaviour and mother-infant contact duration as the outcome variables each showed infant age as the sole significant predictor variable indicating that as infant age increased, maternal behaviours changed as expected above, and mother-infant contact duration decreased. Mothers' interactions with other group members appeared influenced by mothers' associations with their offspring: adult females and juveniles were significantly more likely to be within 1-5 m proximity of mothers as infant age increased. Our data show that mother permissive behaviour, mother-infant contact duration, and proximity are crucial elements to consider when examining wild Southern Pig-tailed Macaque mother- infant relationships and infant independence, similar to what has been observed in captive settings.

In primates, infant dependence on the mother is prolonged compared with most other animals, and the later stages of the mother-infant relationship can vary greatly in terms of maternal permissive behaviours and physical contact, both between and within species living in different settings (Kaufman & Rosenblum, 1969). Physical contact and maternal permissive behaviour have been viewed as critical components of the dyadic mother-infant relationship and significant factors in the attenuation of the bond (Kaufman & Rosenblum, 1969), which gradually leads to increased infant independence. This study focuses on a wild group of Southern Pig-tailed Macaques Macaca nemestrina (Linnaeus). Like other members of this genus, Southern Pig-tailed Macaques live in multi-male multi- female groups that are female-philopatric, where females remain in their natal groups while males leave at sexual maturity (Thierry, 2004). Thierry (2004) places Southern Pigtailed Macaques into the Grade Two category on his four-grade scale of macaque species based on speciestypical patterns of aggression and reconciliation. Grade Two species are characterized by high rates of aggression, high levels of despotism, and relatively low rates of reconciliation (Thierry, 2004). Group size varies between 20 and 80 individuals (Caldecott, 1986), and the dominance hierarchy is stable (Oi, 1990). To date, little is known about this species' mother-infant interactions in the wild, but maternal behaviour has occasionally been studied in captivity. Captive pig-tailed macaques' mothering styles vary between individuals in measures of maternal protectiveness, rejection and warmth (Maestripieri, 1998). Aggression by other adults and previous maternal experience are both important in shaping the captive Southern Pig-tailed Macaque mothering style (Maestripieri, 1998). Captive Southern Pig-tailed Macaque mothers affiliated more with their infants if the mothers experienced hostility from other group members (Maestripieri, 1998). Upon caregivers' attempts to force separation of infants from their mothers, infants were extremely vocal and clung to the mothers; once reunited, all signs of stress and vocalizations ceased (Jensen & Tolman, 1962). Separation increased infant-directed behaviour of the mother, and the infant became less likely to separate from the mother during the early stages of the reunion (Jensen & Tolman, 1962). Mother-infant separation led to more differences in infants' physiology and sleep patterns than did peer separation (Boccia et al., 1989). Lower-ranked mothers categorized in the first two grades in Thierry's (2004) four-grade scale were found to be protective, frequently retrieving infants and restricting infant interactions. In captivity, primiparous pig-tailed macaque mothers often neglected their firstborn (Maestripieri et al.,1997). In the captive environment, pig- tailed macaque mothers initiated the LEN (Lips forward, Ears back and Neck extended) face, a frequent facial expression in this species (Oettinger et al., 2007; also known

as "Pucker" face), when distance between them and their infants increased (Maestripieri, 1996). Infants who received more LEN faces from their mothers did not spend as much time in contact with them compared to infants who received fewer LEN faces (Maestripieri, 1996). Because of this apparent correlation the LEN face was suggested as a means of maternal encouragement of infant independence (Maestripieri, 1996). Rosenblum and Kaufman (1968) designed two studies to compare captive Southern Pig-tailed Macaque mother-infant behaviours with those of other macaque species. They focused on maternal permissive behaviours in Bonnet Macaques Macaca radiata (É. Geoffroy Saint-Hilaire) and Southern Pigtailed Macaques. A female's permissiveness as a mother was measured by scoring three maternal behaviours that tend to change during infant development: restraint of the infant by the mother, retrievals of the infant by the mother, and finally punitive behaviours directed from the mother to the infant (Rosenblum & Kaufman, 1968). Rosenblum and Kaufman (1968) set out to characterize the extent to which a female could be viewed as more or less permissive (both as an individual, and across time) by scoring restraint, retrievals, and punitive behaviours she engages in and how these change as her infant ages. Generally, mothers are expected to restrain and retrieve more and punish less when an infant is younger. As the infant ages, mothers are expected to decrease rates of restraint and retrieval and increase rates of punishment. Their results highlighted physical contact as a critical component in the mother-infant social relationship. In their study, Bonnet Macaques spent a significant amount of time in contact with other members in their group, while Southern Pig-tailed Macaques were not in physical contact with others except when engaged in grooming and mating. Varying contact patterns in adult Southern Pig-tailed and Bonnet Macaques may greatly influence the mother-infant dyadic relationship, and by extension, the social development of the infant (Rosenblum & Kaufman, 1968). Infants of both species initiated breaks in contact early in their lives (Kaufman & Rosenblum, 1969). Mother and infant behaviours were collected separately and scored to reveal the progression of mother-infant interactions over 15 months, which showed a distinct drop in time of maximum separation bouts in Southern Pigtailed Macaques, while the Bonnet Macaques seemed to remain at a constant maximum time (Kaufman & Rosenblum, 1969). In another mother-infant study comparing captive Southern Pig-tailed, Stump-tailed M. arctoides (É. Geoffroy Saint-Hilaire) and Rhesus M. mulatta (Zimmermann) Macaques, Southern Pig-tailed motherinfant pairs spent more time in contact than did Rhesus and Stump-tailed pairs (Maestripieri, 1994). Additionally, Southern Pig-tailed mother-infant pairs showed a gradual decrease over the weeks in the percentage of time spent in contact. Southern Pigtailed Macaque mothers were more protective than were Rhesus mothers and did not encourage infant independence as much as Rhesus mothers did. Maestripieri (1994) observed mothers self-scratching in all three species, which he attributed to maternal anxiety. In all three species, the rate of mother scratching while the infant was away decreased as the infant aged. The greater protectiveness observed in Southern Pig-tailed Macaque mothers compared with Rhesus Macaque mothers may be related to the rate of infant development and the infant's vulnerability in its environment (Maestripieri, 1994). The setting in which a mother raises her young can influence mother-infant interactions. In a study that compared Southern Pig-tailed Macaque mother-infant pairs in two different captive environments, groupraised mothers and infants spent more time in ventral contact and less time completely separated than did caged infants (Wolfheim et al., 1970). Nakamichi et al. (1990) compared individually-housed Long-tailed Macaques M. fascicularis (Raffles) and socially-housed macaque mother-infant pairs from other species. As individually-housed Long-tailed Macaque infants aged, body contact between mother and infant, maternal holding, and infant suckling decreased, while mothers showed increased aggression towards their infants. In a comparison of wild and captive Rhesus Macaque mother- infant pairs, Berman (1980) attributed slight differences in protective behaviours to environment type rather than differences between infants. She found captive mothers were more protective and less encouraging of infant independence than their wild counterparts. In both environments, Rhesus Macaque mothers maintained contact and proximity to infants in the early stages of infant development. Gradually, the mother and infant spent more time out of contact, until a point when the infant was primarily responsible for maintaining contact and proximity to the mother, with an increase in maternal rejections. After several years, captive rhesus mother- infant interactions shifted toward patterns seen in wild mother-infant interactions, characterized by less maternal responsibility in maintaining proximity to her infant. With described variations in physical contact and permissive behaviours existing among captive groups (Kaufman & Rosenblum, 1969; Maestripieri, 1994), and studies on Southern Pig-tailed Macaque and other macaque species showing an impact of setting on mother-infant interactions (Wolfheim et al., 1970; Berman, 1980; Nakamichi et al., 1990), it is important to observe wild Southern Pig-tailed Macaque mothers and infants to develop a more complete understanding of mother-infant interactions in Southern Pig- tailed Macaques. Additionally, no data yet exist regarding the patterns of maternal-infant interactions for the wild Southern Pig-tailed Macaque. We hypothesized that wild Southern Pig-tailed Macaque mother and infant behaviours would change as infant age increased**.** Through the combination and modification of several published

behavioural ethograms used on Southern Pig-tailed Macaques and other related macaque species (Bobbitt et al., 1964; Kaufman & Rosenblum, 1969; Maestripieri, 1994; Schino et al., 1995), we developed two ethograms: one mother-specific and one infant-specific . These ethograms described all mother-infant interactions we saw in wild Southern Pig-tailed Macaque mother-infant dyads. The list of mother behaviours included permissive behaviours initiated by the mother and affiliative contact/non-contact behaviours. The infant ethogram had affiliative contact/non- contact behaviours and a vocalization that commonly occurs when infants are separated from their mothers. Each ethogram included the LEN face (Oettinger et al., 2007) and vocalizations that have been observed from both infants and mothers. We also recorded the proximities of other group members to the mother-infant dyad to test how mothers interact with other group members as the infant ages. We scored proximity into three categories: in contact, We used focal animal sampling (Altmann, 1974) to record the mother-infant behaviours. ED collected focal samples daily, between 07:00 h and 19:00 h, for a duration of 30 min per focal individual sample with a five-minute interval between samples to find the next focal individual. We randomized mothers and infants into a combined sequence, which we edited as the study progressed to account for new births. Subsequent sequences were generated upon completion of the previous sequence with a random sequence generator. ED observed all focal subjects before randomizing the sequence again. If a focal subject could not be found after five minutes, she moved to the next subject in the random sequence list. She then tried to find the missed focal animal for at least five minutes before continuing the sequence. ED recorded the frequencies and durations of both mother and infant behaviours on an iPad mini in the field using Animal Behaviour Pro (University of Kent). The ethogram behaviours were programmed in the application to categorize behaviour frequencies and durations in focal samples. Inter-observer reliability was assessed at the field site with a reliability value of at least 0.85 for animal identity (Martin & Bateson, 2007). ED's intra-observer reliability for ethogram behaviours was assessed using a prerecorded video focal of a mother Southern Pig-tailed Macaque scored at the beginning of the observation period and then each subsequent month (N=4). Intraobserver reliability with ethogram behaviours was at a mean of 87% (range 78-94%). A field study on wild pig-tailed macaques was conducted in West Sumatra, Indonesia, during three periods from January 1985 to February 1987. Two macaque species, the Pig-tailed Macaque (Macaca nemestrina) and Long-tailed Macaque (Macacafascicularis), occur sympatrically in and around the lowland and mountainous forests of the Barisan Range in theKerinci-Seblat National Park in west-central Sumatra. We present and discuss line-transect data on the density,distribution and group size of the two macaques.M. fascicularis was the scarcer, found only in hill dipterocarp and lowland forests. The continuous and extensive conversion of tropical rainforests, home to the world's highest species diversity, is widely believed to be a key threat to the survival of wild populations of terrestrial and arboreal animals, including arboreal non- human primates (Eudey, 1987; Weisenseel et al., 1993; Laurance et al., 2002). It is also now believed that the local numbers of wild Pig-tailed Macaques (Macaca nemestrina) and Long-tailed Macaques (Macaca fascicularis) in Southeast Asia are continuing to decline due to habitat alteration and loss (MacKinnon, 1986). According to IUCN Red List of Threatened Species, M. nemestrina and M. fascicularis are respectively listed as Vulnerable and Least Concern (IUCN, 2008). Both M. nemestrina and M. fascicularis have recently become seriously threatened and fragmented by human encroachment and habitat loss (from illegal and legal logging, traditional and modern crop plantations, land clearance for agriculture and new settlements/ transmigration, forest fires and droughts), as well as hunting for the illegal pet trade. Trading for export by quota for both macaque species still occurs and Sumatra is the main supply source for biomedical research (MacKinnon, 1986; Bowden & Smith, 1992). Presently, there are many cases of land conflict use between macaques and humans and, as a result, both macaque species are regarded as crop pests by farmers. Furthermore, in Sumatra, primary tropical rainforest, especially in the lowlands, have disappeared rapidly (Achard et al., 2002; Kinnaird et al., 2003; Linkie et al., 2004), with most of the land being converted to commercial timber concessions, or cultivated lands and human settlements (FAO, 1981; Holmes, 2001; Jepson et al., 2001). To protect and manage macaque populations and their habitats effectively, the status of macaque populations in protected and unprotected areas must be evaluated continuously (Struhsaker et al., 1975; Wilson & Wilson, 1975a & 1975b; MacKinnon, 1986). Unfortunately, in Sumatra, there has been little effort to date to survey or census primate species, which include gibbons, langurs, macaques, slow lorises, and western tarsiers, either inside or outside of protected areas. The Kerinci-Seblat National Park (TNKS), in the extreme west central region of Sumatra , is one of the Indonesian "treasure houses" of faunal and floral diversity (MacKinnon & Suwelo, 1984). It covers about 1.3 million hectares (Mha) and is the largest national park on Sumatra, and among the largest protected areas in Southeast Asia (MacKinnon, 1986). The park spans four administrative provinces: Jambi, West Sumatra, Bengkulu, and South Sumatra. Primary and secondary rainforests in the national park are occupied by M. nemestrina and M. fascicularis and five other arboreal primate species (Siamang,

Symphalangus syndactylus; Agile Gibbon, Hylobates agilis; Banded Langur, Presbytis melalophos; Silvered Langur, Trachypithecus c ristatus; Slow Loris, Nycticebus coucang), in addition to being an important habitat for many other endangered species. We examined the population status and distribution of macaques in TNKS by direct observation and line transect methods in four different habitat types (lowland, hill dipterocarp, sub-montane and montane forests), at varying elevations.

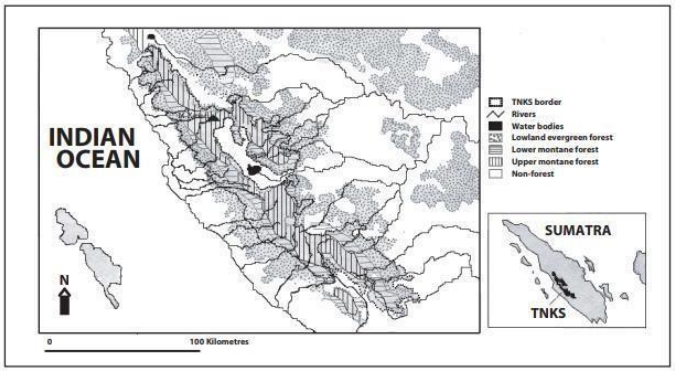


Figure-1

Primate behavior can be responsive to the different ecological pressures associated with different habitats, as well as to the effects of direct and indirect anthropogenic disturbance. The karst forest ecosystem of South Sulawesi (Indonesia) represents one of the few intact forests available for residual populations of the moor macaque, but our understanding of its habitat use is limited. In the present study, this gap in knowledge was addressed by observing the activity and habitat use of two groups of moor macaques and by assessing the suitability of different habitats in the karst forest. Through a fine-scale vegetation analysis of 1 ha of forest in Bantimurung Bulusaraung National Park, we identified the presence of two distinct habitats that differ in terms of forest structure and composition. The karst plain forest (KPF) provided a greater abundance and diversity of food resources than the karst tower forest (KTF). In addition, anthropogenic disturbance was high in the KPF but low in the KTF. Behavioral data collected via group scans indicate that the macaques devoted more time to feeding activities when in the KPF, suggesting an ability to adjust their feeding behavior to meet their nutritional needs. However, the larger of the two groups used the food-rich KPF more than expected, implying that the KTF may represent a valuable refuge for the smaller group, as it is a less risky portion of its home range. The results of this study therefore provide novel information on the ecology of moor macaques and their habitats that can inform conservation planning for remnant populations. Non-human primates (NHPs) can adapt to conditions outside of their natural habitat and climatic ranges but this can be influenced by inherent evolutionary traits or plasticity of species that evolved in diverse environmental conditions. In this study, we investigated how five species of NHPs that have natural distributions across a range of climatic conditions responded to seasonal temperature changes in a captive environment. The activity levels of NHPs were affected by temperature changes over the season, where activity levels were generally reduced at the lower and higher temperature ranges. Species that are naturally found within narrower and warmer climatic ranges, compared to those found in colder environments with wider fluctuations in temperature, showed more marked changes in activity levels in response to temperature changes. In lower temperature conditions, three out of five species showed significantly lower activity levels; whereas in higher temperature conditions, the activity levels of all species did not significantly decrease. The frequency of thermoregulation behaviours was higher, and use of artificial thermoregulatory sources lower, for species that did not substantially adjust their activity levels in different temperature conditions. Our results suggest that NHPs largely retained the evolutionary traits related to thermoregulation, according to the different ambient conditions they evolved in and may have low behavioural plasticity in adapting to conditions outside of their natural ranges.

These results provide insights for improving conservation and captive management and may have implications for understanding NHP resilience to the increasing impact of global climate change. Conversion of primary rainforest to agricultural land causes habitat loss and fragmentation and is a major threat to wild primates worldwide. Conversion of forest to oil palm plantations (Elaeis guineensis) is a particular problem, so it is important to understand whether and how primates use such plantations. Populations of southern pig-tailed macaques (Macaca nemestrina) are declining in Peninsular Malaysia due, in large part, to conversion of primary forests to oil palm plantations. Researchers have observed macaques foraging in plantations but little information is available about how macaques cope with the expansion of plantations into their habitat. We collected GPS data on the home range of a group of wild pig-tailed macaques that foraged in both habitat types in May 2013–May 2015, and compared their use of oil palm plantation and primary rainforest by recording their activity budgets and analyzing their habitat use and diet in both habitat types 4–6 days per week in October 2014–December 2015. The group visited the plantations daily. In 2013–2014, 17% of the group's overall home range core area (0.6 km2) was in oil palm plantations and in 2014–2015, this increased to 28%. However, the macaques spent most of the day time in the forest and always used a sleeping tree in the forest. Macaque activity budgets in the plantation were significantly different from those in the forest. Feeding and foraging comprised a significantly larger proportion of their activity budget in the

plantation, while locomotion, resting, and social behaviors occurred significantly more often in the forest. In both habitats, macaques spent most of their time on the ground and foraged primarily on the ground in the plantation. Of food items eaten in the plantation 85% were oil palm parts, including attached and fallen oil palm fruits and seeds, and flowers. Oil palm plantations serve as additional foraging ground for these macaques, but our results also show that the forest is essential, providing a greater dietary diversity and sleeping sites and allowing resting and social activities. It is not clear to what degree pig-tailed macaque populations can adapt to human- altered environments in the long term. Although our study group used oil palm plantations as regular foraging and feeding ground, pig-tailed macaques are also closely associated with the rainforest habitat, and the protection of natural forest is essential for their conservation. Plasmodium knowlesi is now recognised as a leading cause of malaria in Malaysia. As humans come into increasing contact with the reservoir host (long-tailed macaques) as a consequence of deforestation, assessing the potential for a shift from zoonotic to sustained P. knowlesi transmission between humans is critical. A multi-host, multi-site transmission model was developed, taking into account the three areas (forest, farm, and village) where transmission is thought to occur. Latin hypercube sampling of model parameters was used to identify parameter sets consistent with possible prevalence in macaques and humans inferred from observed data. We then explore the consequences of increasing human-macaque contact in the farm, the likely impact of rapid treatment, and the use of long-lasting insecticide-treated nets (LLINs) in preventing wider spread of this emerging infection. Identified model parameters were consistent with transmission being sustained by the macaques with spill over infections into the human population and with high overall basic reproduction numbers (up to 2267). The extent to which macaques forage in the farms had a non-linear relationship with human infection prevalence, the highest prevalence occurring when macaques forage in the farms but return frequently to the forest where they experience higher contact with vectors and hence sustain transmission. Only one of 1,046 parameter sets was consistent with sustained human-to- human transmission in the absence of macaques, although with a low human reproduction number (R(0H) = 1.04). Simulations showed LLINs and rapid treatment provide personal protection to humans with maximal estimated reductions in human prevalence of 42% and 95%, respectively. This model simulates conditions where P. knowlesi transmission may occur and the potential impact of control measures. Predictions suggest that conventional control measures are sufficient at reducing the risk of infection in humans, but they must be actively implemented if P. knowlesi is to be controlled. Based on previous conflicting reports that the two forms of pig-tailed macaque (northern and southern) exist as separate species, subspecies, or forms, and that their boundary zone lies in Thailand, a survey of the distribution range and morphology of pig-tailed macaques in Thailand was conducted during 2003–2010. We first conducted a questionnaire survey. Questionnaires were sent to 7,410 subdistricts throughout Thailand. We then traveled to 72 of the 123 subdistricts reporting the presence of pig-tailed macaques. However, due to a lack of reports of the presence of free-ranging pig-tailed macaques living south of the Isthmus of Kra, a survey of pet pig-tailed macaques was also conducted during 16–24 September 2011. Furthermore, 35 wild northern pig-tailed macaques inhabiting northern Thailand (13°13′N, 101°03′E) were temporarily caught and their morphological characters were measured and then compared to those of the southern form captured from Sumatra, Indonesia. Although largely allopatric, the ranges of the northern and southern pig-tailed macaques in Thailand were found to have a partially sympatric boundary at the Surat Thani–Krabi depression (8–9°30′N). Morphologically, these two forms were very distinctive, with different morphological characters such as the crown patch, the white color of the triangle above the eyes, the red streak at the external rim of the eyes, pelage color, ischial callosity, tail length and carriage, facial height, and limb length in both sexes, and patterns of sex skin swelling and reddening in females. These differences in morphological characters between the northern and southern forms should help settle the problems of their taxonomy. A comprehensive population study of three monkey species (i.e. Macaca fascicularis, Macaca nemestrina and Trachypithecus obscurus) was piloted to determine the dominant species, population quantity and group size. The survey was conducted between November 2011 and May 2012. The technique adapted for the study was scan sampling method; Population survey showed that there were 23 groups of 508 individuals of three monkeys' species in three states of Peninsular Malaysia (Penang, Kedah and Perak). One-Way Anova test was used to find the significant difference among above three species and to compare their means. Adult males showed a significant difference between two species in terms of population (i.e. Macaca fascicularis and Macaca nemestrina and p-value is $p<0.01$). Adult females, sub-adults and juveniles were found to be significant as compared to Macaca fascicular and Macaca nemestrina which were found $p<0.008$, $p<0.01$ and $p<0.04$ respectively. The infants were found non- significant and Trachypithecus obscurus showed no variation in comparison with other two species. The survey discovered that the Macaca fascicularis was the most dominant species of Peninsular Malaysia. It was noted that the population of Macaca nemestrina was declining and the population of Trachypithecus obscurus was stable

species of Peninsular Malaysia. The rapid and extensive expansion of the agricultural sector, especially planting of oil palms in Sabah are affecting the natural resources considerably. Areas under natural forest cover are being reduced and fragmented resulting in many isolated forest islands located within the converted habitat matrix of agriculture crop. The present study was conducted in a lowland dipterocarp rainforest of Tabin Wildlife Reserve, in Lahad Datu, Sabah, and in the surrounding oil palm plantations that were interspersed with highly degraded isolated forest fragments. The objectives were to assess the values of forest fragments in terms of species compositions, richness and diversity of mammals utilizing the forest fragments, and to assess the effects of retaining forest fragments in oil palm plantation on the small mammal species richness, abundance, diversity and compositions. Twelve trapping sessions using camera traps, direct observations and small mammal traps have been successfully carried out from May 2009 to April 2010. The trapping-sites include one primary forest, three of isolated forest fragments (4.84 ha, 16.07 ha and 26.75 ha) and four oil palm plantations. Overall, 1,913 individual photographs representing 28 species and 15 families of mammals have been recorded by camera trapping. When the mammal species richness were compared between forest habitat and forest fragments, there were 49%, 69% and 65% decrease in the number of mammal species recorded respectively in forest fragments in order of increasing distance from the forest habitat. The forest fragments resulted in the changes of species composition with some species that were recorded as abundant in forest habitat were absent in the forest fragments e.g. Tragulus sp. and Cervus unicolor. However, the Prionailurus bengalensis were found to exist in all forest fragments. For small mammal community, based on 5,220 trap- nights of live trapping, there were 349 animals captured represented by 18 species. The oil palm habitats recorded only six species of small mammal and were highly dominated by Rattus rattus. The percentage of trap success based on all capture events was 6.69%. In conclusion, the forest fragments have provided some habitats for some species of mammals, but both the forest fragments and oil palm habitats harboured very low mammal species richness. However, retaining the forest fragments in the oil palm plantations do not increase the value of the oil palm plantation with respect to the mammal faunas and does not have any effect on controlling the abundance of pest species (rodent) in oil palm plantation. This study discusses the population size of Macaca fascicularis in Penang Botanical Gardens, Malaysia. The scan sampling method was used to observe the groups of M. fascicularis in the gardens. The study was carried out from February 2007 to June 2007. Chi-square test was used to find the correlation of individual appears on the specific area of the gardens. The total number of observations that were carried out during this study was 1134. Among these observation the adult females were observed as 22% ($P = 0.15$), adult males 17% ($P < 0.05$), juveniles 56% ($P < 0.05$) and infants 5% ($P = 0.34$). This study revealed that the population of long tailed macaques is decreasing in Penang Botanical Gardens. In arranging to get better human contact with macaque and at the similar time to keep up a developed macaque inhabitants in Botanical Gardens Penang, there is an imperative requirement for wildlife department to enhance their safety, food availability and predator's threats. Macaca nemstrina has been described as an animal model for acute HIV-1 infection. This animal, unlike most infectedhumans, appears to contain HIV-1 replication. Therefore analysis of HIV-1-specific proliferative and cytotoxic T lymphocyte (CTL) responses following HIV-1 challenge of M. nemestrina may provide information into the role of such responses in both the control of acute HIV infection and protective immunity. Although CD4+ T cell responses to HIV-1 are generally difficult to detect in HIV-1-infectedhumans, early and persistent CD4+ T cell proliferative responses to HIV-1 antigens were detected in all HIV-1-inoculated M. nemestrina. HIV-l-specific CD8+ CTL responses were evaluated in PBMC by stimulation with autologous cells expressing HIV-1 genes, limiting dilution precursor frequency analysis, and T cell cloning. CTL reactive with gag, env, and nef were present 4-8 wk after infection, and persisted to 140 wk after infection. The presence of both CD4+ and CD8 + T cell responses before and after clearance of HIV-1 viremia is consistent with a role for these responses in the successful control of HIV-1 viral replication observed in M. nemestrina. Further studies of T cell immunity in these animals that resist disease should provide insights into the immunobiology of HIV-1 infection. (J. Clin. Invest. 1995. 95:248-256.) Key words: HIV-1 * T lymphocytes – cytotoxic * T4 lymphocytes * Macaca nemestrina. The effect of antioxidant activity on the rate of biological aging was studied in 39 pigtailed macaques (Macaca nemestrina) 7-30 years of age. Scores of seven antioxidant compounds (vitamins C and E, carotenoids, urate, bilirubin, ceruloplasmin, and albumin) were combined to produce an antioxidant variable (AOx) that was tested for correlation with a second composite variable, rate of biological aging (RBA). RBA was formed from seven physiological variables that met a stringent set of criteria as biomarkers of aging. Potential effects of disease on RBA and AOx were excluded by experimental design and by statistical control using a composite index of disease (Dis) that was based on four measures of clinical history and pathology. The study produced three salient findings: (1) there was a significant inverse relation between AOx and RBA (i.e., animals that had high AOx scores had low RBA scores and vice versa); (2) the relation was independent of Dis effects, and (3) there was no significant relation between AOx and Dis

independent of RBA (i.e., the correlation between AOx and Dis was dependent on the correlation of AOx with RBA). These results further validated the RBA variable as a measure of the rate of biological aging and supported the concept that antioxidant activity influences both the rate of biological aging and vulnerability to disease considerable controversy surrounds the relation be- ^—' tween biological aging and disease. Some investigators, citing the greater than tenfold difference in life span across mammalian species, have postulated a genetically tuned aging process that proceeds at different rates in different species and could also vary sufficiently between individuals within species to account for much of the variation in human life span. In that context, increasing vulnerability to disease is regarded as one component of a normal, global aging process (Comfort, 1969; Bourliere, 1970; Hofeckeret al., 1980; Baker and Sprott, 1988). Others, observing that many of the changing features characteristic of aging are readily influenced by disease, have postulated that the impression of a global aging process results primarily from an accumulation of pathological processes which, varying in pattern and rate of development, account for individual differences in life span. Some in the latter group believe that if a global aging process exists, it accounts for so little variation in the human life span that it is of little interest as a factor in the problems of aging humans (Costa and McCrae, 1980; Masoro, 1988). One of the greatest obstacles to scientific resolution of this controversy has been lack of consensus on a definition of aging (National Research Council, 1981; McClearn, 1988). Biologists tend to distinguish between normal and degenerative changes that occur with increasing chronological age. These two dimensions of change have been referred to as "eugeric" vs "pathogeric" (Finch, 1972), "maturational" vs "senescent" (Bowden and Williams, 1984), and "differentiation" vs "aging" (Sohal and Allen, 1990). Clinicians and gerontologists, on the other hand, with a primary interest in disease, use the term "aging" interchangeably with "involutional changes" or "senescence." They tend to include only degenerative changes (Bourliere, 1970; Ludwig and Smoke, 1980) or genetically controlled pathologic changes in the senescent phenotype (Martin, 1978) in the definition of aging. If there is a normal aging process, and if one is to analyze its relation to disease, one must work with a theoretical model of aging that includes both normal and degenerative changes. To this end, we have adopted a definition of biological aging that includes both kinds of change; i.e., aging is "the set of all age-related changes that ordinarily occur in the postpubertal period of males or females of the species, including both maturational and senescent changes" (Bowden and Williams, 1984). A related obstacle to resolving the controversy has been the lack of a phenotypic definition and a measure of the "changes that ordinarily occur"; i.e., variables known to be sensitive markers of the postulated normal aspect of aging (McClearn, 1988). Such a measure is important for at least two reasons. First, to settle the issue of whether disease accounts for a substantial majority of the changing characteristics regarded as aging, one must be able to monitor normal aging and the progress of disease simultaneously. Second, if a global aging process governs the increasing vulnerability to disease, and if one is to evaluate the ability of various interventions to extend life span by retarding the rate of normal aging, one must be able to measure normal aging. Such measurement is particularly necessary for studies in long-lived species for which longevity is not a feasible outcome variable (Bowden, 1988). Since most of the morphologic, physiologic, and behavioral variables one might measure as markers of normal aging can be influenced by disease, it is currently impossible to develop operational definitions of the normal and sense cent dimensions in terms of two separate sets of variables. A proposed solution to this challenge is a battery of tests that measures the rate of aging using a number of variables, or "markers," which represent a variety of physiological systems (Comfort, 1969). Assuming that any particular disease affects only a subset of the markers, the combined measure of aging should not be significantly distorted by changes due to a specific disease (Reff and Schneider, 1982; Baker and Sprott, 1988). A number of human studies have addressed the issue of variability in rate of aging. For the most part they have involved the use of batteries of physical, physiological, and behavioral tests to assess the biological, or functional, age of individuals as distinct from their chronological age (Hollingsworth et al., 1965; Comfort, 1969; Furukawa et al., 1975; Borkan and Norris, 1980a, 1980b; Hofecker et al., 1980; Ludwig and Smoke, 1980; Skalicky et al., 1980; Shock, 1981;Heikkinen, 1982; Voitenko and Tokar, 1983). While such studies have benefited from the combination of multiple diverse measures into a single index, many have dealt inadequately with the confounding effects of normal aging and disease (Costa and McCrae, 1980; Ingram, 1983), and all have suffered from the fact that biological age is at best an indirect indicator of the variable of interest — i.e., the rate of biological aging. To test the relation between circulating antioxidant activity, the rate of biological aging, and disease, it was important to conduct a prospective, longitudinal study in an animal model. Such a study required nonhuman subjects because of the need to obtain frequent measurements of numerous potential biomarkers of aging and to exercise protracted experimental control in order to eliminate diet, evolving culture, and other environmental factors as potential sources of cohort artifact. A primate model was selected because phylogenetic proximity and long life span enhanced the relevance of the study to humans. We developed a battery of tests to measure the rate of aging in pigtailed macaques (Macaca nemestrina), a primate

species with a maximal life span of about 35 years (Bowden et al., 1990). In the course of developing a biological index of aging based on this test battery, we obtained preliminary evidence in female pigtailed macaques consistent with the concept of a global aging process, i.e., when we focused on variables that met strict criteria as biomarkers of aging, we found that the rates of change of those biomarkers were intercorrelated. Animals that showed a rapid rate of change on one of the variables tended to show rapid change on the others as well. The first principal component accounted for 31% of the variance in rates of change of the various markers. A recent study of males of another macaque species, the rhesus macaque (Macaca mulatta), produced comparable findings (Nakamura et al., 1994). Using a similar approach in terms of longitudinal data and linear regression analysis to identify suitable markers and principal component analysis to test for correlated rates of change in the various markers, the authors demonstrated that the first principal component accounted for >50% of the variation. Their findings extended support for the concept of a global aging process because it involved animals of a different species, different sex, and younger adult age group than our earlier study. Furthermore, the markers used to track the rate of aging were different. The present report represents the completion of our effort to validate the index by extending the time period over which measurements were made, expanding the subject pool to include males, testing the sensitivity of the index to variation in circulating antioxidant levels, and elucidating its relation to disease. This effort was based on a theoretical model of the relation of biological aging and disease to each other and to antioxidant level. We regarded antioxidant activity as an inverse index of oxidative stress, which has been postulated to contribute both to the rate of normal aging and to vulnerability to disease (Sohal and Allen, 1985, 1990). The antioxidant variable (AOx) was a combined measure of substances reported to act in different ways as circulating antioxidants, including ascorbate (vitamin C), a-tocopherol (vitamin E), carotenoids, bilirubin, uric acid, albumin, and ceruloplasmin (Ames et al., 1981; Cutler, 1984a, 1984b; Frei et al., 1988, 1989; Halliwell, 1988; Wendel, 1989; Sies et al., 1992). The balance between oxidative and antioxidative processes (Ox/AOx balance) has long been postulated as a determinant of senescence, and thus of life span, in mammals (Harman, 1956; Fridovich, 1975; Cutler, 1984a; Ames and Shigenaga, 1991; Sohal, 1993). According to a series of postulates, senescence results from several effects of free radicals at the molecular and cellular level (Halliwell et al., 1992). These effects include (1) direct action on nucleic acids, which produces deterioration of the genome with a consequent increase in improper gene expression (Adelman et al., 1988); (2) peroxidation of membrane lipids with consequent cellular pathology (Halliwell and Gutteridge, 1985); and (3) accumulation of damaged proteins oxidized by free radicals (Stadtman, 1992). Consistent with the theory are observations that short-lived species such as rodents are genetically tuned to produce free radicals at a high rate and remove them at a low rate, whereas longer-lived species such as primates produce free radicals at a low rate and remove them at a high rate (Cutler, 1984a). Most proponents of free radical theories of aging have adopted the senescence definition of aging. In recent years their theories have found ample and growing support in the rapid accumulation of evidence for the influence of the Ox/ AOx balance on specific diseases. A review by Cross et al. (1987) lists more than 60 clinical conditions in which oxygen radicals are thought to be contributory. Of particular relevance to the theory of aging is the implication of free radical mechanisms in common disorders of the elderly, including cancer, atherosclerosis, parkinsonism, cataracts, renal failure, and ischemic brain damage. A few investigators have suggested that Ox/AOx balance has a direct effect not only on pathogenesis but on the rate of normal aging as well (Sohal and Allen, 1990). That hypothesis was central to the study reported here. We applied the biological index of aging (Bowden et al., 1990) as a measure of the rate of aging to test postulated causal relations among three variables: circulating AOx levels, the rate of biological aging (RBA), and disease (Dis). The suggestion that compounds that protect tissues from oxidative damage reduce the rate of aging (Sohal and Allen, 1990) is represented by an arrow from AOx toward RBA with a negative sign predicted. Bidirectional arrows between AOx and Dis reflect evidence that low antioxidant levels predispose to disease (Cross et al., 1987; Gey et al., 1993) and, conversely, that disease, particularly chronic inflammatory conditions, can lower levels of circulating antioxidants (Halliwell et al., 1987; Frei et al., 1988; Anderson, 1991; Situnayake et al., 1991; Homnick et al., 1993). In either case, high levels of antioxidants are predicted to be associated with low levels of disease and vice versa, so the signs of these postulated influences are negative. Likewise, the relation between RBA and Dis is represented by bidirectional arrows to illustrate the reciprocal hypotheses that deceleration of a global aging process may reduce vulnerability to disease (Comfort, 1969; Bourliere, 1970; Hofecker et al., 1980; Baker and Sprott, 1988) and that disease can alter biomarkers of aging in ways indistinguishable from biological aging (Costa and McCrae, 1980; Masoro, 1988); here the postulated correlations are positive. This model was assessed with composite indices representing seven biomarkers of aging (RBA), concentrations of seven circulating antioxidants (Sies et al., 1992), and the duration and severity of disease (Dis) in 39 pigtailed macaques during a study that covered a 4.5-year time span. The significance of each of the postulated causal relations was evaluated in a sequence of correlational analyses in which we assessed the relation between each pair of variables while controlling for

potential influence of the third variable. The subjects were 60 adult pigtailed macaques (M. nemestrina) maintained in a colony for aging research. At the beginning of the study they ranged from 7 to 30 years of age.The 30 males and 30 females were represented equally in each of three age groups: young adult (7-10 years old), middle-aged (12-19 years old), and old(>19 years old).Birth dates were known for all subjects except the 12 wild born animals in the oldest age group, whose ages were estimated on the basis of weight and dentition when they entered the colony. As these estimates were conservative, none of the 12 animals could have been younger than its assigned age (Short et al., 1987). The subjects were housed in groups of three or four animals of each sex, with at least one animal of each age group in each living group. In addition to the basic study group of 60 animals, a group of 12 animals >14 years old was maintained as a source of substitutes for animals that might die in the course of the study. On the basis of previous spontaneous mortality statistics, we estimated that as many as 20% of the animals might die in the course of the study. Animals that died in the first 2 years of the study could be replaced and sufficient data collected on the substitutes to be included in longitudinal analyses. The animals were housed in rooms 2.5 x 3.7 x 2.8m high, with a light-dark cycle of 12 h on, 12 h off. They received Purina Monkey Chow twice daily (Purina, St. Louis, MO); water was available ad libitum. Other husbandry details are published elsewhere (Short et al., 1987). The health status of each animal was evaluated daily by a member of the research group as well as by animal caretakers trained by the veterinary staff to recognize signs of illness. Data were taken from animals only when they were healthy, thereby ensuring that all measurements were normal and uncontaminated by disease. All measurements were made between 0800 and 1100 h, thus minimizing circadian variation. A prospective, longitudinal, correlational study was designed to test for relations among three composite variables: AOx, RBA, and Dis. RBA, as a rate index of change over time, was based on longitudinal measures taken over a 4.5- year period (May 1985 to November 1989). AOx, as a trait index, was based on several measures concentrated in the middle years of the study. Dis, as a potential covariate of RBA and AOx, was based on retrospective examination of clinical and pathology case records. RBA index. — To identify the best 5-10 variables for inclusion in the RBA index, we evaluated 44 physiological variables selected from 72 variables tested in an earlier cross- sectional study. In that study, each variable was evaluated for feasibility of measurement in M. nemestrina, reliability on repeated measurement, and correlation with age in a cross- sectional design (Short et al., 1987). In the present study the number of variables was reduced by selection according to four criteria: (1) a significant within-subject rate of change with increasing chronological age in both females and males; (2) heavy loading on the first principal component in a principal component analysis of variables that met the first criterion; (3) a relatively stable rate of change per year regardless of chronological age; and (4) physiological independence vis-a- vis other variables in the panel of potential markers. For data collection, animals were removed from their home rooms and placed in individual holding cages for 24-h urine collection and 12 h of fasting. Then they were lightly anesthetized with ketamine (7-10 mg/kg) and transferred to the laboratory, where blood samples for serum chemistries and antioxidants were obtained and physical measurements were recorded. For complete blood count and immunological specimen collection, fasting animals were lightly anesthetized in their home rooms and then taken to the lab for sample collection. Because about 10 measurements are required to assure reasonable power in the estimate of a single regression coefficient (Cohen, 1977), every effort was made to acquire at least 10 measurements of each variable on each animal distributed over the duration of the study. Each variable was measured at intervals of at least 4 months and not more than 6 months. Physical measures and samples for blood chemistry and creatinine clearance determinations were obtained semiannually. Blood for complete blood count and immunology determinations was obtained at 4-month intervals. The methods of collection and analysis are described elsewhere (Short et al., 1987; Bowden et al., 1990, 1994) and summarized in Table 1. AOx index. — The AOx index was based on concentrations of seven circulating antioxidants: ascorbate (vitamin C), ot-tocopherol (vitamin E), carotenoids, urate, total bilirubin, albumin, and ceruloplasmin. Bilirubin and albumin were measured at least six times over the course of the study. Tocopherol and carotenoids were each measured twice at 6- month intervals. Ascorbate was measured twice with a 9- month interval. Ceruloplasmin and urate were each measured three times at 6- month intervals. Serum for bilirubin, albumin, and ceruloplasmin determinations was prepared in a routine manner. Determinations of a-tocopherol and carotenoid were made from specimens that had been maintained in darkness from the time of collection until analysis. Blood for ascorbate and urate determinations was combined with an equal volume of perchloric acid (50 mmol/L) before centrifugation, and the supernatant was frozen for analysis. Ascorbate was measured by high-performance liquid chromatography (HPLC; Lee and Hamernyik, 1982). The samples, which had been maintained at- 70 °C, were thawed and, together with control material and standards, were kept on ice prior to analysis. Control material, consisting of 100 jig/ml ascorbic add in 3.5% bovine serum albumin, was made up daily and was measured to be within 2 standard deviations of the long-term daily mean value. Standards were different dilutions of the same material to produce a two- point calibration curve. The supernatant was injected into a HPLC

(Waters Chromatography, Milford, MA) reverse-phase column (300 x 3.9 mm, 10 [x,m Bondavak) developed by a formic acid mobile phase. An electrochemical detector (Bioanalytical Systems, West Lafayette, IN) was used with a strip chart recorder (Omniscribe, Houston Digital Instruments, Houston, TX). An internal standard, 3,4-dihydroxybenzylamine hydrobromide, was used in quantification of ascorbic acid. Total carotene was measured by spectrophotometry (Nino and Shaw, 1976). Serum was added to ethanol to disrupt the carotene- protein association. Bilirubin and other xanthophylls remained in the ethanol layer, while the carotene was extracted into a hexanexhloroform mixture. The solvent layer was removed and the carotene was measured by its absorption at 455 nm and calculated by using a predetermined factor. Ceruloplasmin was measured by single radial immunodiffusion. Serum was applied to a cylindrical well in a thin layer of 2% agarose containing an anti-human ceruloplasmin produced in animals (NOR-partigen immunodiffusion plate; Behring Diagnostics Inc., Somersville, NJ). Antigen was quantitated from the diameter of the precipitin ring when diffusion ceased (24 or 48 h), which indicated the combination of the applied antigen with antibody at equivalence endpoint when the antibody present in the matrix was sufficient to react with the amount of antigen applied to the well. a- Tocopherol and retinol were measured by HPLC (Bierrei et al., 1979). They were extracted from plasma in a total lipid fraction and injected into a reverse-phase column (Waters HPLC with Bondapak C18 column) that was developed by isocratic elution with methanol and water. A dual ultraviolet detector (Waters model 440) was used with detection at 313 nm for retinol and 280 nm for a-tocopherol. Retinolacetate and a-tocopherol acetate were added to the specimen as internal standards, allowing calculation of retinol and atocopherol concentrations. Uric acid was measured from plasma by HPLC from the uric acid peak height as it appeared on the ascorbic acid analysis chromatogram (see above). The internal standard, 3,4-dihydroxybenzylamine hydrobromide, was used for quantification of uric acid in comparison with uric acid standards. Dis index. — The Dis index was based on four measurements of disease and pathology obtained from computerized records collected by Primate Center veterinary and pathology staff over the duration of the study. Two variables represented health status: the number of days the animal was under veterinary treatment during the 4.5-year span of the study (DAYS), and an average of the severity of disease (SVR). The clinical severity of each episode was rated as mild (+ 1), moderate (+ 2), or severe (+ 3) on the basis of veterinary records. The mean severity of illness was calculated for each animal as the mean of the severity scores across episodes. Two other variables concerned the status of each animal's intestine, colon, kidney, and liver, the organs most commonly affected by lesions. The most severe diagnosis in each organ was rated on a scale of 0-3 where 0 meant there was no lesion at necropsy or the animal was still alive. These tissue damage scores were summed for intestine and colon together (IPATH and for renal and hepatic tissue together (RHPATH). Intestinal/colonic lesion scores were calculated separately from renal/hepatic lesion scores because intestinal disease is by far the major cause of morbidity and mortality in macaque species (Hird et al., 1984), and we needed to be able to analyze their relation to AOx and RBA either together with the renal/hepatic lesions or separately. Data Analysis Since measurements were taken only if an animal was healthy, not all measurements on all variables were obtained on all animals. To ensure that only data from the most healthy animals were included, only those that met minimal criteria for number of observations were included in the comprehensive analysis. For the AOx index, a variable had to be measured at least twice. For the RBA index, a variable had to be measured on at least six occasions over a 2.5-year period — i.e., about 7% of the 35-year maximum life span of the species (Bowden and Williams, 1984). A total of 39 animals, 25 females and 14 males, provided data sufficient for analysis. These animals were reasonably well distributed over the total age range: 16 young adults, 12 middle-aged adults, and 11 old adults. Fewer males than females met criteria for inclusion because the reserve pool from which animals were selected to replace those that died included almost no males, and the lack of measurements due to morbidity was somewhat more common in males. The first step in data analysis was preprocessing of the RBA, AOx, and Dis measurements to maximize the content validity of the indices. RBA was computed for each animal by a procedure described in detail elsewhere (see biological index of aging, or BIA, in Bowden et al., 1990). Briefly, a slope measure was computed for each variable in each animal by least-squares regression to provide a longitudinal estimate of the individual rate of change. The mean rate of change across animals (mean slope of the regression lines) was tested in each sex separately by Mest to determine whether the change for the variable differed significantly ($p < .05$) from zero. Only variables that yielded significant change scores in the same direction across animals in both sexes, 14 in number , were included in subsequent analyses. Age, sex, and age-sex interaction were still seen as possible sources of undesired variance for an RBA and were therefore removed statistically from the slope measures by cross-sectional multiple regression. Because one female was much older than the others and hence could potentially produce undue leverage in correlational analyses, a histogram was plotted for each set of residual scores that resulted from the removal of age and sex, and the scores were recoded into four to six categories. The recoding was done by a modified Winsor estimation process. The original scores were linearly

transformed to a new scale and rounded to the nearest interval. The highest and lowest values were placed in the extreme intervals. The result was a symmetrical and uniform distribution with interval scale properties and good metric properties. This transformation reduced the precision expected of the data, but eliminated potentially undue influence of outliers on the correlational analyses. Principal component analysis was performed on the 14 variables to determine the signs of their loadings on the first component. This completed the analysis of the individual variables to determine which of them should be included in the RBA index. The seven most informative markers were selected on the basis of their loadings on the first principal component and the other criteria described above. Calculation of the animals' rates of aging proceeded as follows. The values of all of the animals on each variable were standardized by conversion to z-scores — i.e., the mean across animals was subtracted from each animal's value — and the difference was divided by the standard deviation to produce a z- score. The signs of the z-scores were reflected such that all loaded with a positive sign on the first principal component. Each animal's RBA was calculated as the mean of its z-scores on the seven markers. Additionally, coefficient a was computed from the reflected scores. AOx, like RBA, is a composite variable for which there is no established mode of calculation. In addition to the group of circulating antioxidants measured in this study, a number of noncirculating enzymatic and metal-binding antioxidants located in specific tissues contribute to the organism's total antioxidant capacity, and their activity levels may interact with the circulating antioxidants. Which antioxidant serves as the first line of defense varies depending on the location and source of the oxidative challenge. Because of these complexities there is no generally accepted combined measure of antioxidant activity suitable for measuring the latent variable AOx. Our AOx measure was patterned on that of the RBA measure, except that all of the antioxidants measured were included in the index. A mean score was computed for the concentration of each of the seven AOx variables in each animal across occasions. The means were then recoded into three to five categories to ensure that any outliers would not unduly influence subsequent estimates of the relations between variables. A principal component analysis was done on the seven variables to determine their signs of loading on the first component. The further derivation of the AOx index from the seven variables for each animal was obtained following the same steps as for the RBA — i.e., reflected zscores were averaged across variables for each animal. The four Dis scores were standardized in a manner similar to that used for the AOx and RBA variables: measures were weighted equally by setting the mean of each to 0.0 and the standard deviation to 1.0. The four standardized scores for each animal were then summed to form a composite measure of Dis, and an a coefficient was computed from their correlation matrix. In the case of these four variables, no reflection of signs was indicated. A series of partial correlational analyses was used to test specific hypotheses regarding the relations among the AOx, RBA, and Dis variables. For example, to test the association between AOx and RBA independently of the potential common influence of Dis, a partial correlation between AOx and RBA was computed with variance due to Dis removed statistically (Cohen and Cohen, 1983). Data were managed and analyzed on an IBM-XT personal computer. Application software included MR-TOD (Retriever Data Systems, Seattle, WA) for data management and SYSTAT (Evanston, IL) statistical software for linear regression, partial multivariate correlational analysis, and principal component analysis. Within-subject regression and interpackage translation routines were written in-house. Marker selection and calculation of the RBA. — Of the 44 variables that were considered as candidates for RBA marker variables, 14 met the criteria for inclusion as a biomarker of aging in both sexes. Measurement reliability was >.87 for all variables and >.98 for the majority. [Measurement reliability was calculated as the test-retest correlation coefficient (Nunnally, 1978) of duplicate measurements of physical characteristics and blind duplicate specimens for chemical analyses.] The 14 variables are listed in Table 2, together with the range of numbers of measures taken per animal and the mean percentage of change per year across all animals of each sex. (The estimated slope for each animal was divided by the mean value of its measures and multiplied by 100; the mean percentage of change across animals was then computed.) In the course of the longitudinal study, the number of measures per variable on each animal ranged from 6 to 25. Six variables increased over time and eight decreased. Themean percentage of change ranged from -7% per year for albumin to + 17% per year for the females' activated suppressor T cells. Multiple regression analysis showed that none of the 14 markers had a significant age, sex, or age X sex interaction effect. Reduction in the number of variables to achieve minimal redundancy and maximal physiological range of markers and maximal content and construct validity was achieved as follows. Exclusion of redundant variables eliminated the serum electrolytes sodium, potassium, and carbon dioxide, which correlated with serum chloride. Serum albumin and total bilirubin were excluded because as antioxidants they were included in the AOx index and would create a correlational artifact if included in the RBA index as well. Blood urea nitrogen and platelet count were excluded in the interests of construct validity because they showed the least correlation with the first principal component, which we regard as representing the rate of biological aging (Bowden et al., 1990). The remaining seven markers — rectal body temperature, percentage of bone cortical area, activated suppressor T cells, serum chloride,

serum calcium, mean corpuscular volume, and white blood count — represented the least redundant, most informative sample of the domain of physiological markers. The internal consistency reliability (coefficient a) of the seven RBA variables, computed from their intercorrelations, was .73. This exceeded that in our earlier analyses of data from females only (Bowden et al., 1990) and was greater than the .70 usually anticipated in the early stages of developing an index (Nunnally, 1978). Of the seven slope measures contributing to the RBA, four — rectal body temperature, percentage of cortical area of bone, and serum chloride and calcium concentrations — decreased with age and were negatively related to the RBA . Animals that decreased on these measures faster than their cohorts were judged to be aging faster biologically. Activated suppressor T cells and white blood cell count increased with age and correlated positively with the first principal component, indicating that the faster these biomarkers increased the faster the animal was aging biologically. Mean corpuscular volume decreased with age but had a positive loading on the RBA. Thus, those animals whose corpuscular volume decreased less rapidly were judged to age faster biologically. The correlations of these markers with the first principal component accounted for 38% of the variation in the, biomarker values among animals . Antioxidant values and calculation of AOx. — The mean concentrations of the seven AOx compounds are Presented. Six were within or very close to the human range. As expected, uric acid was present in a concentration about 40 times less than the human mean . To determine whether there was reliable covariation among concentrations of the seven antioxidant variables, a correlation matrix was formed and the seven compounds were entered into a principal component analysis. The first principal component for this set of marker variables was AOx. Loadings on AOx ranged from -.69 to + .72. The internal consistency reliability of the AOx composite score was .67. The correlations of the individual markers with the first principal component. The Dis index was based on the four marker variables: days in treatment, severity of illness, intestinal pathology, and renal-hepatic pathology. The internal consistency reliability of the Dis composite measure was .78. A total of 80 episodes of illness were treated, i.e., an average of two episodes per animal during the 4.5-year course of the study. The range was 0 episodes in 10 animals to 7 episodes in 1 animal. The average treatment period was 19 days. This low level of illness in a group composed largely of middle-aged or elderly animals confirmed the expectation that by restricting the analysis to animals that had at least six measurements on every variable during at least 2.5 years of study we had focused the analysis on animals that were basically healthy. The most common diagnoses in the 80 treatment episodes were diarrhea with or without dehydration and weight loss (45%) and infections secondary to oral disease or trauma (14%). More serious conditions included wasting (14%) or death (16%) due to intestinal, renal, and/or hepatic disease. The remaining treatments were for trauma (8%) and conditions such as anemia and dermatitis (3%). Intestinal pathology, consisting largely of moderate to severe chronic diffuse nonsuppurative inflammation of the small intestine and/or colon, was the primary histopathologic finding in seven (54%) of the animals that died. Chronic glomerulosclerosis,mesangioproliferative glomerulonephritis, and inflammation of the kidney were the most common moderate to severe lesions in three animals that died with a primary diagnosis of renal disease (23%). One animal died with a primary diagnosis of hepatic disease, i.e., fatty infiltration with mild amyloidosis. Renal and hepatic lesions of moderate severity, particularly chronic interstitial inflammation of the kidney and hepatic amyloidosis, were commonly seen in animals with a primary histopathologic diagnosis of intestinal disease. As predicted, AOx, RBA, and Dis were significantly intercorrelated, and the correlations were in the directions anticipated: Dis and RBA were positively correlated and both correlated inversely with AOx. Partial correlational analysis was used to test further relations among the three indices . To test the hypothesis that Dis might influence both AOx and RBA, and thus account for the observation that high levels of AOx were significantly associated with low RBAs and vice versa, the correlation between AOx and RBA was tested with variance due to Dis partialled out. The correlation remained significant ($r = -.365$; $df = 33$; $p < .025$). This finding was consistent with the hypothesis that Dis was not responsible for the correlation between AOx and RBA, but that AOx influences RBA directly. To test whether AOx activity might influence both RBA and Dis and thus account for the correlation between the two, that correlation was tested with variance due to AOx partialled out. In this case the positive partial correlation between RBA and Dis was not significant ($r = .244$; n.s.), a finding consistent with the hypothesis that AOx can influence both RBA and Dis, either directly or one through the other, sufficiently to account for the positive correlation between the two. Finally, to differentiate between those two possibilities, we tested whether the effect of AOx on Dis might be mediated through its effect on the RBA. This was done by a semipartial correlational analysis in which the correlation between AOx and Dis was conducted with the variance due to RBA removed from the Dis variable. In this case, again, there was no significant correlation between AOx and Dis ($r = -.266$; n.s.). This suggested that the association of high levels of AOx with low morbidity and vice versa was exerted predominantly through the effect of AOx on RBA. We had attempted to control for the potential effect of chronological age on RBA by statistical extraction of its effect on the individual markers of aging. The very low correlation between Age and RBA

indicated the success of that procedure. The effect of Age on Dis, however, had not been controlled for and, as expected, was large . To confirm that a residual chronological age effect did not account for the correlation between RBA and Dis, a partial correlation was computed in which the effect of Age was removed. The correlation between RBA and Dis remained significant ($r = .42$; $df = 36$; $p < .01$), confirming that chronological age did not account for the relation between the two. In summary, the results of sequential partial correlational analysis were consistent with the hypothesis that individual differences in AOx account for inverse individual differences in RBA which, in turn, account for individual differences in Dis . To rule out the possibility that subclinical intestinal disease, the predominant disease of macaques, might have contributed to aging-like changes in the RBA markers, the analyses were repeated with only episodes and signs of intestinal disease included in the Dis variables. The rationale for this analysis was twofold. First, a subclinical syndrome of mild dehydration, infection, and undernutrition resulting from chronic low-grade intestinal disease might not be sufficiently detectable to exclude animals from testing but might affect variables such as serum electrolytes, white blood cell count, activated suppressor T cells, and even body temperature and cortical bone thickness, which were not segregated into the Dis variable but rather made a "residual" contribution to the RBA variable. Second, in the analyses described above, inclusion of other kinds of disorder, viz., renal and hepatic disorders, might mask a correlation between RBA and Dis owing to chronic intestinal disease alone. Thus, in a second analysis, only disease episodes and pathology findings involving signs of intestinal disease were included. Exclusion of nonintestinal signs did not alter the outcome of the analysis. The results of the study were consistent with the basic model . The basic bivariate correlation analysis revealed significant correlations among AOx, RBA, and Dis, and the directions of the correlations were as predicted. The signs of the correlation coefficients between Dis and the other two variables were consistent with common hypotheses based on the geriatric research literature — i.e., the inverse relation between AOx and Dis was consistent both with evidence that low AOx levels predispose to disease and with evidence that disease can lower the levels of circulating AOx. In either case, high levels of AOx would be predicted to be associated with low levels of Dis and vice versa. Likewise, the relation between RBA and Dis appeared as a significant positive correlation, consistent with the reciprocal hypotheses that deceleration of a global aging process may reduce vulnerability to disease and that disease can alter biomarkers of aging in ways indistinguishable from biological aging. Further analyses in which the relations between the variables taken two at a time with influence of the third variable partialled out provided additional information about the predominant directions of influence among the three variables. The finding that the relation of AOx to RBA remained significant when the effects of Dis were removed supported the concept that AOx contributes significantly to the regulation of RBA. That, coupled with the finding that no significant relation between AOx and Dis remained when the effect of RBA on Dis was removed, suggested that the effects of AOx and RBA on Dis are greater than coordinated effects of Dis on either AOx or RBA. The most parsimonious summary of the results is that AOx contributes directly to the regulation of RBA and contributes to the regulation of Dis directly and/or indirectly through its effect on RBA . Correlations of the three primary variables with Sex and chronological Age (Table 6) were also consistent with the basic model and experimental design. The positive correlation between Age and Dis was consistent with both conventional wisdom and the basic model . The lack of correlation between Age and AOx was consistent with the view that AOx is a trait variable — i.e., a characteristic of the individual which does not change greatly in the course of adult life. The significant correlation between Sex and Age was expected on the basis of the greater age span in the female sample, and the lack of correlation between Sex and any of the three experimental variables indicated that the greater range of ages in the female sample was unlikely to have influenced other results. The mechanisms whereby antioxidants might exert protective effects at a molecular level have received considerable experimental attention (Frei and Ames, 1992; Halliwell et al., 1992). The antioxidants exist in different concentrations in different body compartments, both at the organ level (e.g., blood vs cerebrospinal fluid) and at the tissue/cell level (e.g., aqueous vs lipid environments), where they can exert their effects in several different ways. A major basis for the hypothesis that variation in an AOx set-point might account for individual differences in RBA was the observation that species with high blood concentrations of circulating antioxidants, particularly a-tocopherol, carotenoids, and uric acid, have longer life spans than species with lower concentrations (Cutler, 1984a). Thus, in this study we focused on the circulating, small molecular extracellular antioxidants ascorbate, a-tocopherol, carotenoids, bilirubin, and uric acid, which act largely by direct scavenging of oxidants (Frei and Ames, 1992). Albumin, a larger protein molecule, can also act as a scavenger or, like ceruloplasmin, as a metal or iron-compound-binding, anticatalytic agent against destructive oxidative reactions. The antioxidants that act as scavengers are used up in the process and must be resupplied if concentrations are to be maintained. Since some can be regenerated by others (Frei and Ames, 1992), a genetically based mechanism to maintain any one at a given level might draw others in the circulation to a correspondingly high or low level. The positive loadings seen in Table 3 may have resulted from such a mechanism. To that extent genetic control of only

one or a few of them would be necessary to determine the sum of all. Uric acid is one of the circulating antioxidants that correlates with maximum life span across species (Ames et al., 1981; Cutler, 1984b). Humans, the most long-lived of the primates, have particularly high levels because they lack uricase, the terminal degradative enzyme present in monkeys and other mammals (Ames et al., 1981; Schreiber et al., 1986). It has been proposed that across species the absolute concentrations of the circulating antioxidants, or an interaction between their concentrations and the metabolic rate (as an index of the rate of production of oxidants), is the primary determinant of RBA (Cutler, 1984a, 1984b). The finding of substantially lower levels of uric acid in macaques, which have a shorter life span than humans, is consistent with that concept. Uric acid was particularly interesting in this study because, while its concentration correlated with concentrations of the other antioxidants and with the first principal component of AOx, its correlations were inverse to the others. Higher concentrations of uric acid were associated with lower concentrations of the other antioxidants and loaded negatively vis-a-vis AOx. Thus, while the difference in mean values between humans and macaques supports the concept that uric acid contributes to the difference in life span between the species, the significance of the reverse relation within species may be quite different. Within species, uric acid may play a different homeostatic role. The inverse correlation between uric acid and the other circulating, nonenzymatic antioxidants that we found in the macaques appears to exist in humans as well (Situnayake, 1991; Olinescu et al., 1992). It may reflect a homeostatic mechanism which maintains AOx within certain limits. Increases in other circulating antioxidants may result in downregulation of uric acid concentration, and decreases may lead to upregulation. Such a mechanism would tend to stabilize total antioxidant activity under variable conditions of oxidant challenge, at the same time limiting variability in the rate of aging among individuals of the species. There is direct evidence that uric acid has a stabilizing influence on ascorbate in human blood (Sevanian et al., 1991); it can protect ascorbate not only as a scavenger (Ames et al., 1981; Cutler, 1984b) but also as a stable, noncatalytic binder of iron or iron compounds that would otherwise catalyze the oxidation of the ascorbate. As an endogenous compound whose concentration depends primarily on the rates of synthesis and degradation of purines (Wyngaarden, 1982), uric acid is theoretically susceptible to regulation through a balance of synthetic and degradative enzymatic activity. Thus, while the lack of uricase and the resultant high level of uric acid relative to that in macaques may be one reason humans live three times longer than macaques, the variation in the set-point of the homeostatic mechanism that regulates urate concentration in individuals may regulate differences in the rates of aging of individuals within the species. Individuals with higher concentrations of nonurate antioxidants would age more slowly than their conspecifics with lower concentrations, but the magnitude of the difference from other individuals would be limited by a suppression of their uric acid levels. While this study provides further evidence in support of the free radical theories of aging, such theories will remain unproved (Sohal, 1993) until it can be shown that extended reduction of the Ox/AOx balance reduces the rate of biological aging. Up to now there is some evidence that the administration of antioxidants can retard disease related to oxidative stress (e.g., Fahn, 1992) but does not extend the life span. Antioxidant mechanisms appear to be regulated such that interventions to increase activity by administration of one antioxidant may be offset by reductions in others (Sohal, 1993). This does not mean that the balance of oxidative and antioxidative processes — i.e., oxidative stress (Sohal and Allen, 1990) — is not the primary determinant of the rate of biological aging. It does mean, however, that the homeostatic mechanism that maintains a low level of oxidative stress in slowly aging individuals and a higher level in rapidly aging individuals may be robustly resistant to set-point manipulation. It may not be easy to perform the experimental manipulation of AOx necessary to test the hypothesis. The seven variables selected as biomarkers of aging in this study differ from those used in our previous study (Bowden et al., 1990) and from those used by Nakamura et al. (1994), who applied a similar approach to estimate the rate of biological aging in groups of rhesus macaques. Only one of the seven markers, serum chloride, was the same as in our previous report. The primary reason a different set of markers emerged as best in this study was that further constraints were added to the criteria for acceptable markers: a marker had to show significant change in both sexes. Thus, variables that increased linearly with chronological age and loaded strongly on the first principal component in the previous allfemale analysis but did not show a similar pattern in males were excluded. Redundant markers were strictly eliminated. Other possible factors were the opportunity to select from a wider range of potential markers and extension of the longitudinal data collection period from 21 -30 months per animal to 30-54 months per animal. The small number of animals compared with the number of subjects in human epidemiological studies may also have limited the number of markers identified. Many factors could explain why the best biomarkers of aging that emerged in this study differed from those reported by Nakamura et al. (1994). First, Nakamura et al. restricted their definition of biological aging to "processes that reflected declining vitality and increased vulnerability of the organism," thus favoring variables that are sensitive to diseases of the aged. Our set of potential markers, on the other hand, included variables that would reflect normal aging as independently of disease effects as possible. Second, Nakamura et al. gave

heavy weight to agreement between the results of cross-sectional analyses and longitudinal analyses in selecting markers. We have found the results of cross-sectional analyses to be so infrequently predictive of longitudinal changes in individual animals (presumably owing to cohort and selective mortality effects) that we have not placed great weight on such agreement in selecting markers (Bowden et al., 1994). Species of Macaca have been variously separated into several species groups according to different authors (Fooden 1976; Delson1980; Fa 1989; Groves 2001). On the basis of male genitalia morphology, Fooden (1976) classified the macaques into four species groups: the silenus-sylvanus group, the fascicularis group, the sinica group, and the arctoides group. Pig tailed macaques were classified under the silenus-sylvanus group. The silenus group which consists of Macaca silenus, Macaca nemestrina and Macaca nigra forms a monophyletic clad and this group is considered the most diverse lineage within the macaque genus. Based on morphological characteristics, such as pelage color, pattern, and tail morphology Fooden (1975) recognized three subspecies of pigtail macaques (nemestrina, leonina, pagensis), although he was not fully conclusive about the subspecies status of the three forms (Gippoliti 2001). Groves (1993) confirmed Fooden's conclusion but later recognised M. pagensis (the Mentawai macaque) as a full species (Groves1997). Until recently, M. nemestrina included M. leonina and the nominal form as subspecies. Based on genetic evidences (Evans et al. 1999; Morales and Melnick 1998; Tosi et al. 2000) and sexual swelling distinctions, Groves (2001) proposed full specific treatment for Macaca leonina and Macaca nemestrina. They are now recognized as two species of pig tailed macaques, Macaca nemestrina, or the southern pig tailed macaque and the northern pig tailed macaque Macaca leonina. The two species are known to hybridize in a small area of southern peninsular Thailand, as well as on the islands of Phuket and Yao Yai (Groves 2001). The macaques are characterised by their short, 'pig-like' tail, with the tip partially resting on the rump. The macaque possesses a relatively long, uniformly agouti golden-brown coat, with markings confined only to the brown crown, buff-coloured cheek whiskers and the red streak extending from the outer corner of each eye. Macaca leonina and Macaca nemestrina differ in morphological characters such as the crown patch, the white color of the triangle above the eyes, the red streak at the external rim of the eyes, pelage color, ischial callosity, tail length and carriage, facial height, and limb length in both sexes, and patterns of sex skin swelling and reddening in females. In particular, there are differences in the sexual swellings in the two species (Gippoliti 2001). Sexualskin swelling during and after the menstrual period is particularly developed in the macaques of the silenus- sylvanus group (Fa 1989). In M. nemestrina the swelling forms a "continuous pillow like mass" while in M. leonina (like silenus) the development of the swelling is mainly limited under the tail. Gippoliti (2001) observed that adult female Macaca leonina in captivity demonstrate a highly developed subcaudal swelling resembling that of Macaca silenus. The northern pig tailed macaque (Macaca leonina) is distributed throughout northeastern India (Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland and Tripura), eastern Bangladesh, Cambodia, southern China (southwestern Yunnan), Lao PDR, Myanmar, Thailand (from about 8°N and including adjacent islands), and central and southern Vietnam (Boonratana et al. 2008). In India it is found towards north of the Brahmaputra river (Groves 2001). Recently it has also been recorded from Namdapha National Park in Arunachal Pradesh (Chetry et al. 2003). In central and northeastern Myanmar, it has not been recorded between 20 and 25°N except on the coast at Arakan. The precise taxonomic boundary between M. leonina and M. nemestrina is not well defined. There are populations of the two species found on either side of the distribution limits in the Isthmus of Kra, but many of these populations might be due to release by humans. In Vietnam, there are historical records from the Nghe An province, but there is uncertainty as to whether the species was ever found north of this province in areas of human settlements (Boonratana et al. 2008) It occupies tropical evergreen and semi-evergreen forest, tropical wet evergreen forest, tropical moist deciduous forest, coastal forest, swamp forest, low elevation pine forests (in Lao PDR and China) and montane forest, including degraded forests. In China the species occupies elevations between 50- 2,000 m (Molur et al. 2003; Choudhury 2003). In Lao PDR and Vietnam the species is associated with lowlands, usually below 500 m. Pig tailed macaques are diurnal. While northern pig tailed macaques (Macaca leonina) are predominantly arboreal (Gippoliti 2001, Feeroz 2012), the southern pigtails (Macaca nemestrina) spend most of their time on the ground, and ascend to trees to escape predators or to sleep (Caldecott 1985). Because of their large group size, pigtail macaques often split up into foraging groups to decrease direct competition for fruit at feeding sites (Crockett & Wilson 1980; Caldecott 1986). Their arboreal time is also divided between different canopy levels, with most time spent in the middle canopy (47.4%), then the lower canopy (33.8%), and finally the upper canopy (10.4%) (Rowe 1996). The day range length varies between 825 and 2964 meters, depending on weather conditions and seasonal fruit availability (Caldecott 1986). In Bangladesh the day range of M. leonina was reported to vary between 950 to 3340 meters (mean=1746±527.8) (Feeroz 2012). Although M. leonina are chiefly arboreal, the macaques of the Nambor and Garampani Wildlife Sanctuaries of central Assam come down to the ground and frequent the highway in search of sugarcane left over by the elephants (Choudhury

2010). Pigtail macaques are primarily frugivorous, with 74% of their diet consisting of fruits, but they also consume a wide variety of food including insects, seeds, young leaves, leaf stems, dirt, and fungus (Crockett & Wilson 1980; Caldecott 1986). They also feed on nestling birds, termite eggs and larvae, and river crabs (Rowe, 1996). Northern pigtails (Macaca leonina) were reported to spend most of their feeding time in the middle canopy (Feeroz 2012). While foraging they may divide into groups of 2-6, but remain in close contact with other groups through vocalizations. Southern pig tailed macaques being ground foragers also raid crops particularly during rainstorms, when most farmers are inside (Crockett & Wilson 1980). In a study on captive pig tail macaques, it was found that they preferred food rich incarbohydrates and fructose over food that is low in these nutrients and they also tend to prefer foods that are low in zinc (Laska 2001). Macaques live in female-bonded, multimale-multifemale groups (containing both adult males and females, with their dependent offspring) with the adult sex ratio biased towards females, who are usually philopatric. Females form kin-bonded subgroups (matrilines) within their natal groups and most males usually disperse at sexual maturity (Thierry 2007). The basic social unit of pigtails consists of an average of 20-40 individuals. The male to female sex ratio is about 1:8 (Caldecott 1986) and mean ratios of immature to mature animals (adult and adolescent, juvenile and infant) is 1:1.2 (Oi 1989). Most adult males live as peripherals or solitaries. Caldecott (1986) suggested the mechanism for this 'shedding' of males from groups in pig-tails involves displays and antagonism by the group-living adult males towards adolescent and other adult males. Pigtail juveniles and adolescent males associate with one another. Males leave the natal group at the age of 5-6 years either roam independently as solitaries or try joining another group first as the lowest-ranking male and then work their way up through competition with other males (Oi 1989). Males of 5-6 years of age live as temporal troop individuals, not yet as complete solitaries. After repeating temporal stays, they may become complete solitaries at an older age (Oi 1989). Immigration into and emigration from the troop is mainly restricted to males, and is never observed in females (Oi 1989). The dominant male pig tail is responsible for group defence and control of internal disturbance and aggression (Sackett, Oswald, and Erwin 1975; Erwin 1976). They are known to be more sociable toward humans and less aggressive than other related species (Sussman et al. 2013) and can also be easily trained to perform certain tasks (picking coconuts) (Crockett & Wilson 1980). Sackett, Oswald, and Erwin (1975) found that all-female groups in captivity exhibited more aggression than groups containing an adult male. Female pig tails are known to spend more time in social interactions, including agonistic as well grooming behaviour (Bernstein 1972). The species is reported to make use of a large range of affiliative behaviors (Maestripieri 2005; Oi 1990a, b; Thierry 2000). Females both groom as well as receive more grooming than male pig tails (Bernstein 1972). Highranking males tend to associate with females by grooming and agonistic alliances, while low-ranking males do not get this chance, and frequently suffer from allied attacks involving females and juveniles (Oi 1989). Pig-tail macaques are non-seasonal breeders. Estrus in females occurs throughout the year, with a peak from November to January (Oi 1990). The males compete for mating partners. Wild troops show a tendency towards mate monopolisation by a few top-ranking males. The tendency to mate monopolisation by pig tail males was also reported from a laboratory study (Tokuda et al. 1968). Sexual access by subordinate males to the females in estrus is restricted by the presence of dominant males. The monopoly of copulation by the dominant males is achieved by the presence of only a few females in estrus with conspicuous sexual signs at a given time. The smaller the number of females in estrus, the more conspicuous is the mate monopolisation by the dominant males. However, as the number of females in estrus increases, the rates of copulation by the subordinate males also increase. Females solicit even the lower-ranking males and tend to copulate with many males and sometimes they may reject attempts by top ranking males to copulate. Hence, female choice counteracts the monopolistic tendency of the dominant males. The mating relationship is rightly called a superficial promiscuous state, because of the inability of the dominant males to exert mate monopolisation at times when too many females are in estrus. When a female reaches sexual maturity at 3 years of age, she starts cycling and might present herself to males with her anogenital swelling for reproduction. It has been studied in captivity that females showing delayed swelling are usually of lower weight (Erwin and Erwin 1976). In females, an external indicator of ovarian condition is provided by the swelling of the perineal or sexual skin (Bullock et al. 1972), particularly prominent during the middle 12 days of the 32 day cycle (Mitchell 1979). Laboratory studies have shown that pig-tails are multi mount ejaculators (Tokuda et al. 1968; Nadler and Rosenblum 1973). Pigtail males are known to use elaborate non-copulatory mounting for conciliatory purpose more frequently than other macaque males (Oi 1989). Among males, non-copulatory mounting tend to be performed in the direction from the subordinate to the dominant, while among females, it tends to be in the reverse direction; and grooming is an important form of conciliation among females. Male infant pigtails attain independence from their mothers earlier and to a greater extent than female infants and mothers play a major role in developing the male's greater independence (Mitchell 1979). Adult males are indifferent to infants but will protect them when they are attacked (Mitchell 1977). Although there is no

precise information available on the total number of individuals in India or Myanmar, but populations have been reported to decline in both the countries (Boonratana et al. 2008). A group density of 0.07 individuals/km² was recorded in Namdapha National Park, India, by Chetry etal. (2003). In China, the species' population is estimated to be less than 1,000 individuals (Zhanget al. 2002). No information is available on the status of the population, but is thought to be declining rapidly. A small isolated population occurs in Bangladesh, whose habitat is degrading rapidly, thereby leading to the continuous decline in the number of mature individuals in the country (Molur et al. 2003). In Thailand the populations have been reported to be stable. During the last 30-35 years more than 30% of the population has been reported to decline in countries like India, Bangladesh, China, Vietnam and Myanmar (Boonratana et al. 2008). In Lao PDR and Cambodia, there has been perceptible decline in population size but the rates are close to or lower than 30% (Boonratana et al. 2008). In most of the countries, the species is predicted to decline at a rate higher than 30% over the next three generations. The species is threatened by habitat disturbances such as selective logging; timber and firewood collection for making charcoal; building roads, dams, power lines; and deliberately setting fires. Deterioration in habitat quality due to the loss of fruiting trees and sleeping sites through monocultures and plantations, selective felling, and a subsequent increase in the canopy gaps lead to forest fragmentation and soil erosion Other threats include hunting and trade for food, sport and traditional "medicine", and accidental mortality due to trapping occurs. There is a local trade for bones, meat for food and the live animals as pets (Molur et al. 2003). Habitat loss and poaching are the major threats in India and Bangladesh. There has been a reduction in forest cover in Assam by over 10% in two years between 2001 and 2003 (Forest Survey of India 2003). In Lao PDR, Vietnam and Cambodia, hunting for food and trade is the primary threat, but as a predominantly lowland species habitat loss likely is also a major threat to the species. In Thailand, the males of this species are exploited for picking coconuts by the industry. Habitat loss and disturbance are the major threats in China and Myanmar (Boonratana et al. 2008). Northern pig-tailed macaques are known to occur in several protected areas of Bangladesh, China, India, Myanmar, Thailand and Vietnam (Boonratana et al. 2008). The species is listed as Vulnerable A2cd in the IUCN Red list of threatened species (2008), as the species has declined by at least 30% over the past 30-36 years (three generations) due to hunting and habitat loss. It is listed under CITES Appendix II. In Bangladesh it comes under Schedule III Bangladesh Wildlife (Preservation) (Amendment) Act, 1974. In China it is listed in Category I under the Chinese Wildlife Protection Act (1989) and as Schedule II in India under the Indian Wildlife (Protection) Act, 1972 (Chetry et al. 2003) amended up to 2002. Moreover, the Central Zoo Authority (CZA), India identifies Pig tailed macaque among the 73 critically endangered species for planned and coordinated conservation breeding in Indian zoosThe global captive population of Pig tailed macaque consists of 187 individuals (70 males, 85 females, 32 unknowns) and are currently maintained in captivity in 37 institutions across four regions- Africa, Asia, North America and Europe (ZIMS data until 2014). This record includes individuals housed in Indian zoos and they have been pooled together as Macaca nemestrina however the Central Zoo Authority website refers to it as Macaca leonina. This Pig tailed macaque National Studbook records all the individuals from the Indian captive population for this species. Pig tailed macaques have been kept in Indian zoos since 1962 and the living population is housed at 7 institutions. Pedigree data was collected by means of mailed questionnaires, zoo visits and from the websites of CZA and ZIMS (Zoological Information Management System). Questionnaires were sent to 14 institutions housing Pig tailed macaques in India, requesting information for each captive specimen. Data was entered in the Single Population Analysis and Records Keeping System (SPARKS v 1.66) (ISIS 2004) and subsequently exported to population management program PMx v 1.2 (Ballou et al. 2010). Data was exported from SPARKS and used as input files in PMx for further analysis. Further visualization and analysis of pedigree data was performed using the program Lineage v 1.06 (Pollak et al. 2001). The development of effective population management plans is dependent on data availability in terms of knowledge of complete pedigree records and dates of events. The availability of data with reference to the Pig tailed macaque captive population in Indian zoos is summarised in A total of 14 institutions have housed the species since 1962; however data was available from only 11. These data limitations in conjunction with the small population size and the larger percentage of animals being of wild origin without birth date estimates limited demographic analysis of the population. Birth date estimates were unavailable for more than 70% of the wild-born individuals and complete parentages were recorded for 22 out of 35 captive births. The historical population includes a total of 79 individuals (37: 34: 8) that have been recorded in Indian institutions from 1962 to 2013. Of these, 44 (22: 21: 1) were wild-born and 35 (15: 13: 7) were captiveborn individuals. The sex based population trends are depicted in Figure 4. During the first 40 years the population remained small with a median of 10 (9.36±3.31) individuals only, the numbers increased to a median of 39 (36.36±8.89) individuals from 2003 to 2014. Another strategy represents the population trends based on origin of animals over the years. The population reached a maximum of 44 in 2013. Another indication that the increase in the population from 2000 to

2013 was due to both acquisitions from the wild as well as births in captivity. During the period 2000-2013, there were 20 acquisitions from the wild and 26 captive births. The current population contains 44 individuals (20: 18: 6), of which 22 are captive-born (7: 9: 6) and 22 wild-born (13: 9: 0). The sex ratio of the living individuals is 1.3:1. The ages of 33 (14: 13: 6) individuals are known. The age distribution suggests that 8 individuals (24.24%) are in their pre reproductive ages (0-3 years); 18 individuals (57.57%) are in their reproductive ages (3-13 years) and 6 individuals (18.18%) are in their post reproductive ages. This suggests that with appropriate management interventions the species is capable of rapid growth required to provide a demographically stable captive population. Of the total 79 individuals data for life table analysis was available for only 48 individuals. And of the 48 individuals, 35 were captive-born and the rest were wild born for which only birth date estimates and not exact birth dates was available. Death dates were available for all the 15 dead individuals from the group. Age distribution of these 48 individuals showed that for each age class the number of individuals varied between 0 to 7 individuals. Since, for a life table analysis to provide valid results the data should be sufficient in terms of total numbers, age structure of the population (at least 30 individuals in each age class) and precise birth and death dates, the results of the life table for Pig tailed macaque population could not be used for drawing conclusions about the population. A total of 44 (22: 21: 1) individuals are of wild origin with a mean of 0.78±1.3 captures per year. The sex ratio of wild born individuals was 1.05:1. Birth date estimates were available for 13 individuals and the median age at capture for these individuals was 0.3 years (1.3±1). The median time spent in captivity by all wild born individuals was 10 years (11.6±7.9). At present the captive population of Pig tailed macaques has reached a total of 44 individuals and is being maintained by a combination of acquisitions from the wild as well as captive births. The captive population had few individuals in the breeding pool (only 30% of the population has bred at least once) highlighting poor overall breeding success in the population. In the current population however; out of a total of 44 individuals 22 individuals (10 males, 12 females), or 50% of the current population, have bred at least once. Of these, 5 were captive born and 17 were wild born individuals. Among the females that had bred, 7 (58.3%) had one off spring each and 5 (41.6%) had two or more off springs. There were a total of 35 (15: 13: 7) births with a mean of 0.67±1.3 births per year. The sex ratio of captive births was 1.15:1. Out of 35 births, parentage for 22 was known, for the remaining births either or both the parent's identification was unknown. From a total of 42 wild origin individuals (excluding one that was lost to follow up and another that escaped) 19 have reproduced, 18 died without breeding and are yet to contribute to breeding. Of the 42 wild-born individuals (excluding one that was lost to follow up and another that escaped) 19 have reproduced, 18 died without breeding and 6 are yet to contribute to breeding. The wild-born individuals to have reproduced spent a median of 6 (7.36±3.9) years before they reproduced for the first time in captivity, with about 26% (5 individuals) to have spent more than 10 years in captivity before breeding. Similar trends were also observed for the potential founders – they have been housed for a median of 7.5 (8±4.6) years in captivity. The fecundity of females of wild origin was the highest with 11 out of the 21 females breeding (slightly more than 50%) this declined in the F1 generation in captivity where only 3 of the 12 females reproduced (25%) while in the F2 generation the lone female reproduced. The wild born individuals to have reproduced spent a median of 6 (7.36±3.9) years before they reproduced for the first time in captivity, with about 26% (5 individuals) to have spent more than 10 years in captivity before breeding. Similar trends were also observed for males. The lowered reproductive rates over generations suggest deficiencies in husbandry probably in the nutritional aspects as literature suggests that low weights of females result in delayed age of sexual maturity and lowered reproductive potential. The cumulative birth and mortality rates of the captive population are depicted in Figure 8. A total of 33 (17: 14: 2) deaths were recorded. Of these 21 were wild born and 12 were captive born individuals. The mean annual mortality rate was high during 1977-1981 and 1992-1996 and has decreased in the following years. The population shows a higher death rate than the birth rate throughout its history indicating that the growth in population observed is a result of inclusion of wild origin animals rather than captive births. This suggests that the population is currently demographically unstable as the population is unable to produce enough individuals to maintain itself. Gene diversity is the principal measure of genetic diversity in populations and ranges between 0 and 1. Current retained gene diversity is 0.9374. This signifies that the population has retained 93.74% of the founder gene diversity. However; a large part of this may be ascribed to the fact that the current population comprises of 50% wild origin individuals. The mean inbreeding coefficient is the average of the inbreeding coefficients among the living individuals, weighted by the % known for each individual. Over 93 % of the living population shows an inbreeding coefficient of zero. For the remaining ~7% either the inbreeding coefficient is not known or have relatively high inbreeding coefficient of 0.25. The mean inbreeding coefficient of the population is 0.0156.The results of the analysis carried out suggests the need for improvements in husbandry practices in management of zoos as suboptimal conditions have been associated with reduced reproductive potential.

III.CONCLUSION

The current husbandry practices followed by the holding zoos need to be assessed and shortcomings identified and addressed. The species shows female philopatry and lives in troops comprising multiple males and females. Accordingly the social organization of the species must be maintained in captivity. It may not be possible to capture entire troops for conservation breeding, however; with appropriate socialization process the troop organization can be recreated in captive environments. This can be achieved by pooling together the single/pairs of animals housed across the various holding institutions to form viable social groups with appropriate socialization process. With appropriate management interventions and modifications in the husbandry practices the species is capable of the rapid growth required to provide a demographically stable captive population. Once the population has achieved demographic stability the genetic diversity can be managed by regulation of mating choices and inclusion of additional founder animals as required. This will require all holding zoos to maintain complete records of all events in each individual's life history.

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