Preface

This supplemental issue in the Natural History Journal of Chulalongkorn University (*NHJCU*) is published to compile the original papers, review articles, short notes, and abstracts, which were presented at the symposium entitled "International Symposium on Southeast Asian Primate Research: Biodiversity Study from DNA to Ecosystem" held from 17th to 20th of October 2005, in Bangkok. In this symposium, papers on such diverse aspects concerning primates as paleontology, archeology, biogeography, genetics, morphology, ecology, social behavior, veterinary science, environmental enrichment for captive animals, and preservation biology, were presented. Special session was devoted to the primate diseases and pathology. Although the text materials were not included here, four workshops were also held in the lecture room, primate facilities (Primate Research Unit, Chulalongkorn University), and in the fields at the Non-hunting area of Khao Kra Puk-Khao Tao Mo (on stump-tailed macaques) and at the Phra Nakhon Khiri National Park (on long-tailed macaques), Petchaburi Province. Although macaques are well-known and familiar primates in SE Asia and most of them are not in the state of endanger as other primates, such as Douc langur, their local populations are threatened their survival and/or genetic properties by various human activities. It is probable that their majority of biodiversity will soon be lost before their evolutionary history can be elucidated. From exchanging ideas and knowledge on the diverse aspects of SE Asian primates, hopefully, we can construct the future perspectives of primate studies, the measures to preserve their biodiversity, and the international network of persons interested in primates. We therefore hope the readers of this issue of NHJCU will share these ideas.

We thank to Assoc. Prof. Somsak Panha for his consideration to publish papers presented in the symposium in the NHJCU.

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BIODIVERSITY STUDY FROM DNA TO ECOSYSTEM

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Biodiversity of Macaques in Southeast Asia

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ABSTRACT. **-The genus *Macaca* is composed of 19 species which show enormous biodiversity in characters and each of the species also exhibits great variation. The constructed evolutionary history of rhesus macaques reveals the processes by which such biodiversity was produced, that is, the origin, expansion and shrinking of distribution, variation produced by isolation or climate variation, divergence of offspring species, and introgression with other species. Macaques selectively utilize forest environments and their distribution reflects the forest type. To ensure the maintenance of macaque biodiversity, we should take measures now to preserve macaque populations living under various environmental conditions and, of course, their habitats.**

KEY WORDS: Southeast Asia, Macaques, Biodiversity, Conservation

INTRODUCTION

The Genus *Macaca* arose about 7-8 million year ago (MYA) in Africa, and they deployed over Eurasia from around 5.5 MYA (Delson, 1980). They then expanded their distribution east to South Asia, Southeast Asia, East Asia, and finally to such remote islands as Japan, Taiwan, and Sunda. Along with migration and expansion of distribution, so many species diverged that taxonomists generally consider three or four species groups under the genus level (Fooden, 1980). This specific diversity was produced by branching into and within species groups, coupled with the different ecology of each species (Fooden, 1982). Each of the living macaque species shows geographical variation in morphology, and some of them exhibit characteristics striking enough to be classified as different subspecies. Based on genetic variation, various phylogenetic hypotheses have been advocated (e.g. Tosi et al., 2003). For macaques, the evolutionary pathways are considered to have been influenced by geophysical factors, such as alternating glacial-interglacial periods or changes in continental shape and relief due to plate-tectonic movement. This should be reflected in the diversity of biological characters in species but research on these topics is only possible if local populations are well preserved.

At present, the value of forest environments for humanity is receiving greater attention and those with great biodiversity that can supply various kinds of resources should be used sustainably. Macaques prefer such forests, and they show different preferences of forest types depending on the species. As their population density depends on the quality of forest, macaques may be regarded as a good indicator of forest condition. Macaques presumably contribute to maintain the forest ecosystem via mechanisms such as seed dispersal.

In the present study, I discuss the importance of biodiversity studies and propose

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methods to assist in the preservation of macaques.

EVOLUTIONARY SCENARIO OF RHESUS MACAQUES

I briefly outline here the evolutionary course in rhesus macaques (*Macaca mulatta*) to illustrate how present day macaque biodiversity has been produced.

The evolution of rhesus macaques has been reconstructed mainly from morphological (e.g. Jiang et al., 1991) and genetic analyses (e.g. Tosi et al., 2000; 2003) on living animals because of the paucity of fossil materials (Fooden, 2000). The rhesus macaque is a member of the *fascicularis* species-group, along with long-tailed (*M. fascicularis*), Japanese (*M. fuscata*), and Taiwanese (*M. cyclopis*, Fooden, 1980), so the evolution of these other members should be also be taken into consideration. Fooden (2000) described the evolutionary scenario of rhesus macaques in his monograph as follows:

1. Divergence from the common ancestor with long-tailed macaques: Origin of rhesus macaques (>0.4 MYA): The ancestor of rhesus macaques is considered to have diverged from a common ancestor with the long-tailed macaques at around the base of Indochinese peninsula. In general, rhesus macaques deployed to more northern areas than the long-tailed macaques. According to this geographical distribution, tail length, the most striking of the morphological species specific traits (relative to head and trunk length, in %), differs between the two species, ca. 110% in long-tailed macaques and less than 90% in rhesus macaques.

2. Northern expansion of distribution in rhesus macaques: The proto-rhesus macaques dispersed to the north in the continent of eastern Asia (mainly in China). In accordance with latitude, they exhibited a clear geographical cline in tail length, the southern-most population (15-degree N) had the longest (ca. 90%) and the northern-most one (40-degree N) had the shortest tail (ca. 30%). The former further migrated to the Japanese islands through northeast China and the Korean Peninsula, whereas groups from the southern-most population migrated into Taiwan. These insular populations subsequently evolved independently into endemic species as the two islands became separated by sea. The Japanese population came to have a still shorter tail (ca. 15%), whereas the Taiwanese population preserved its longer tail (ca. 80%) because of a warmer climate.

3. Southern retreat of northern populations deployed in China during the last glacial period (around 18 thousand years ago, KYA): Rhesus populations deployed in northern China retreated to the south in the last glacial period. At the glacial peak, around 18KYA, rhesus macaques were distributed as far north as 25-degree N, to the west around Bangladesh, to the east coast facing at the South China Sea, and to the southern central region of the Indochinese peninsula. Thus, the distribution had become half of the distribution maximum, but the area was still wide and geographical variation was found. The western populations tended to have longer (ca. 45%) and the eastern ones shorter tails (ca. 30%). As the southern populations deployed in localities bordering the distribution of long-tailed macaques had longer tails (ca. 60%), they were considered to be hybrids between the two species.

4. In the post-glacial period rhesus macaques rapidly expanded their distribution, both in eastern and western populations, to attain their present huge distribution area: Eastern populations rapidly expanded their distribution to the north and west in continental China. Western populations, too, rapidly expanded to the west, to cover the northern two thirds of the Indian sub-continent, Nepal, Pakistan, and Afghanistan.

Genetic analyses are providing details to augment the evolutionary scenario described above. Based on sequence analyses of mtDNA, which was obtained from rhesus macaques of various origins in Asia, Smith and McDonough (2005) reported that the eastern populations appeared to be composed of a number of sub-populations with longer divergence times, whereas western samples showed similar

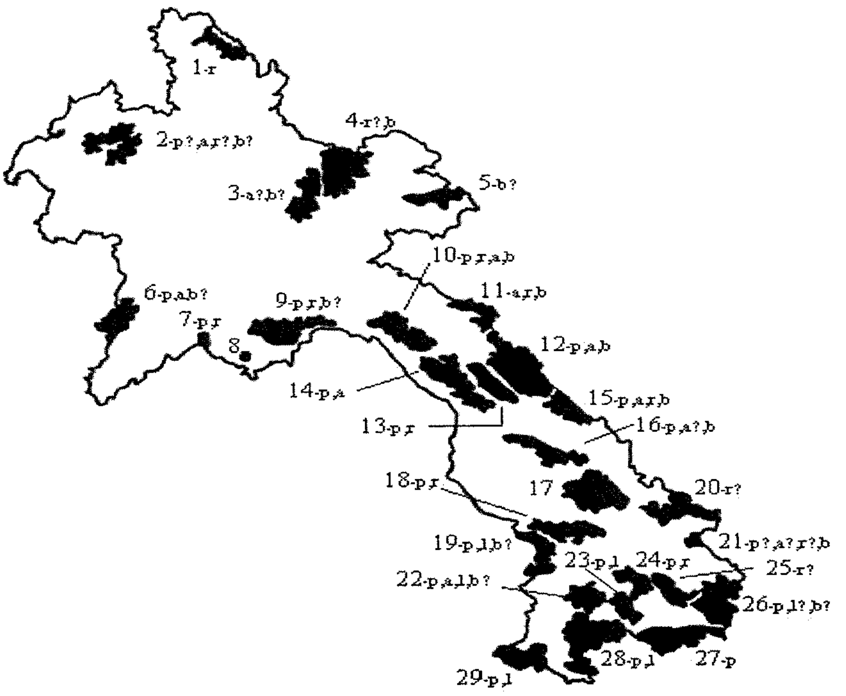

FIGURE 1. The distribution of macaques in National Biodiversity Conservation Areas (NBCA) and other localities (modified from Duckworth et al., 1999). Numbers in the diagram indicate the NBCA and letters attached to the number show the macaque species observed. Questionable species are indicated by the "?" attached to letters. NBCA: 1. Phou Dendin NBCA, 2. Nam Ha NBCA, 3. Phou Louey NBCA, 4. Nam Et NBCA, 5. Nam Xan NBCA, 6. Nam Phoun NBCA, 7. Sangthong District, 8. Houay Nhang Nature Reserve, 9. Phou Khaokhoay NBCA, 10. Nam Kading NBCA, 11. Nam Theun Extension PNBCA, 12. Nakai-Nam Theun NBCA, 13. Nakai Plateau, 14. Kahmmouan Limestone NBCA, 15. Hin Namno NBCA, 16. Phou Xang He NBCA, 17. Dong Phou Vieng NBCA, 18. Xe Bang-Nouan NBCA, 19. Phou Xiang Thong NBCA, 20. Xe Sap NBCA, 21. Phou Ahyon, 22. Dong Hua Sao NBCA, 23. Bolaven Southwest PNBCA, 24. Bolaven Northeast PNBCA, 25. Phou Khathong PNBCA, 26. Dong Amphan NBCA, 27. Nam Ghong Provincial PA, 28. Xe Pian NBCA, 29. Dong Khanthung PNBCA. Macaque species: p= pigtailed (*M. nemestrina leonina*), a= assamese (*M. assamensis*), r= rhesus (*M. mulatta*), l= long-tailed (*M. fascicularis*), and b= stump-tailed (*M. arctoides*).

sequences to each other. Different genetic studies (Hayasaka et al., 1996; Tosi et al., 2000) have shown that the eastern population is closer to Japanese and Taiwanese macaques than to the western populations, meaning that the two rhesus macaque populations diverged before the above-mentioned stage-3. Taking the social structure of macaques, male dispersal and female philopatry, and the matrilineal inheritance of mtDNA into consideration, it can be supposed that female genes preserve the ancient divergence event, and that the genes conveyed by dispersing males gave rise to common morphological characters both in eastern and western populations. The rapid expansion of distribution and frequent gene flow may account for the paucity of geographical variation in rhesus macaques.

Further details will be added to the above-mentioned evolutionary scenario by the future studies, which will reflect past environmental events. Forest habitats, for example, were considered to have shrunken in glacial periods in the area of the Indochinese peninsula due to a cooler, drier climate. Macaques would have survived in these forest patches located in mountains or along river banks, the so-called "refugia" (Eudey, 1980; Koenig et al., 2003), but the influence of isolation within these refugia on the evolutionary history of macaques is left for future study.

There is one particular question of interest in this scenario regarding whether the longer tails in southern rhesus macaque populations are the result of introgression from long-tailed macaques (Tosi et al., 2002) or the retention of an ancestral trait. If the former is the case, other characters specific to long-tailed macaques should be evident in those populations, and we can also determine if any rhesus characters occur in long-tailed macaques inhabiting the area close to the boundary zone (15-20 degree N). The phylogenetic relationships between two such closely related macaque species are an important subject to be studied (Hamada et al., *in press*).

OUR BIODIVERSITY SURVEYS ON MACAQUES

Aiming for a nationwide determination of distribution and the present status of macaques, we are conducting surveys in Vietnam, Laos, Thailand, Myanmar, and Bangladesh as follows:

1. Literature survey (Fig. 1)
2. Questionnaire survey: We send authorities (head of the village or persons with a rich knowledge of wildlife) brochures explaining the macaque species and containing questionnaires inquiring as to the <presence/absence>, <species>, <density, <damage caused by macaques>, <counter-action to macaque damage>, and so on. We are conducting these surveys either nationwide or in selected localities.
3. Extensive trip survey: From the result of questionnaire survey, we visited some of these localities where macaque troops were thought to occur. Consulting with authorities or via direct observation, we collected information about species distribution, troop number and troop size, present status of troops, etc.
4. Catch and release survey: By sampling from monkeys temporarily caught, we can record morphometry and take blood or fecal samples, which enables us to describe the morphological characteristics of each of troop, and conduct genetic analyses.

The data will be analyzed to determine the ecological and human factors affecting distribution and present status. We are using GIS (Geographical Information System) to combine our data and the geographical information from the satellite or aerial photos of vegetation.

These surveys do not always offer us detailed ecological parameters such as troop numbers and population sizes. This is especially true in the case of National Parks or Conservation Areas, which have wide areas with complicated topography and few human inhabitants (informants). Population census studies are necessary, such as line-census (Steinmetz et al., 1996), DNA analysis of feces or other obtainable biological materials, or remote morphometry, all of which require longer periods of field work and, therefore, the co-operation of park authorities or area managers, and support from government, NGO or NPO specialized in wildlife.

The natural environments for macaques are variously preserved in the five countries surveyed (Fig. 2). We found that priorities in preservation efforts tended to be given to such rare and endangered wildlife as tigers, elephants or snub-nosed langurs. This is understandable, of course, but I must also stress the importance of macaques deployed over wide areas because a survey on them would contribute to the preservation of various and large-scale environments. Macaques are a good indicator animal of habitat condition, as will be explained. In the very near future, my colleagues and I will report on a recent nationwide survey of distribution and the present status of macaques in the five countries listed.

ECOLOGICAL CHARACTERISTICS OF MACAQUES AND IMPLICATIONS FOR THE PRESERVATION OF THEIR BIODIVERSITY

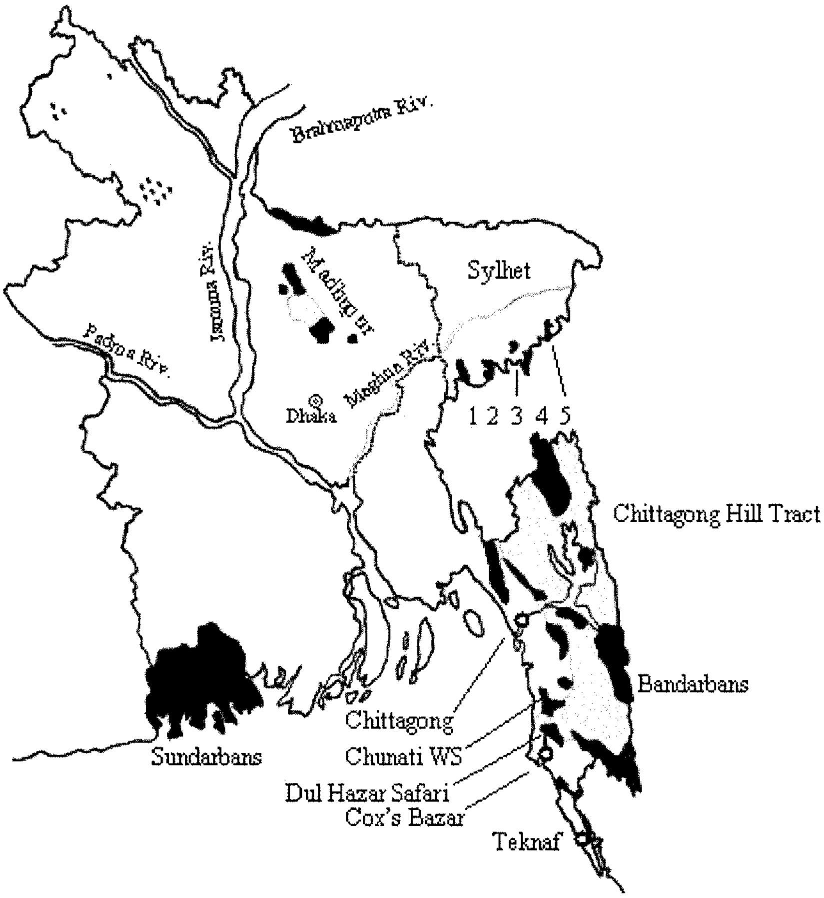

FIGURE 2. The distribution of Forests in Bangladesh and the National Parks (NP) and Wildlife Sanctuaries (WS). Painted parts are the continuous forests, and dotted parts are the mixed forest with bush (modified from Khan & Ahsan, 1986). Small-scaled forests are scattered in Sylhet Division, North-eastern Bangladesh: 1. Schacchori, WS, 2. Rema-Kalenga WS, 3. Lawachera NP, 4. Forest inside Tea-Estate, and 5. Patharia forest inside the Tea-Estate.

The importance of macaque preservation relates to their ecology. Macaques live in social troops of 10 to up to 1000 animals. To sustain the troops of these medium sized mammals, forest habitats need to have a proportionately wide area of floral and faunal diversity and richness. In general, macaques show different vegetation preference according to species, for example, evergreen and non-evergreen forests (Fooden, 1982). Macaque species also have different preferences of canopy substrate levels, starting from the difference between arboreal and terrestrial. Although the ranges of ecological requirements overlap considerably among species, the optimum differs with species. Therefore, macaques may be regarded as an indicator animal characterizing the forest habitat (Borries et al., 2002).

In the five countries where we are conducting surveys, there are five species of macaques in common, which differ in ecological requirements, and they are distributed either allopatrically or sympatrically. Rhesus and long-tailed macaques do not share habitats, in general, because of their similar requirements, whereas pig-tailed and long-tailed macaques can share habitats via separation of substrate level, that is, terrestrial (pig-tailed) and arboreal (long-tailed). Macaques also differ in their tolerance to the human impact. There are species that absolutely require primary forest (*M. silenus*), whereas others can exploit disturbed forests or human settlements (*M. mulatta, M. fascicularis, M. radiata*). The latter are often known as commensal or weed species.

Though the degree to which macaque activity impacts on the vegetation and forest environment has not been thoroughly studied, they do disperse seeds and thereby assist in the reproduction of food plants. The detailed ecological role of macaques is a subject for future study.

Macaques are close to humans, not only phylogenetically, but also in various biological characters. They have been and will continue to be used for biomedical studies in which biodiversity is of importance, for example, sensitivity to Malaria infection is known to be species (or local population) specific. People, especially Asians, feel intimacy with macaques and enjoy observing them. Habituated and even wild macaques are found without difficulty because they live in groups and are often noisy. Macaques are sometimes regarded as sacred animals (e.g. legend of Hanuman) and are included as one of the twelve zodiacal animals. In SE Asia, people traditionally lived in forests and had a strong relation with wildlife. They hunted macaques for foods, traditional medicines, or other use (meat, skin, or gallstone "bezoar stone"), but they did not wipe them out.

Although no one wants macaques to become extinct from any given locality, people tend to consider that there are no alternative ways to prevent the disappearance of macaques due to such destructive activities as indiscriminate logging or the construction of dams and roads. This is partly because alternative ways have not been considered.

PRESENT TREND: PRESERVATION OF MACAQUES AND THEIR HABITATS

There is a trend at present for people of all over the world to share in the recognition that natural forest resources should be sustainably used. These resources may take diverse forms; commercial products like timbers, foods or traditional medicines, influences on water circulation and global climate, and places for recreation or education.

The forest environment used to be regarded as "exterior" to economic activity and it has consequently been destructively used. Such uses were often driven by politics, explosive population increase, or economies pursuing short-term profits. However, people have begun to realize that such use only turned a resource rich environment into an infertile one, and that local people had not received any benefits, only costs. The forest environment should be regarded instead as a crucial aspect of economic activity. Now is the time for people to consider wise and sustainable ways of resource exploitation. This trend is typically expressed by the "Biodiversity convention" ratified by many countries and regions. Similarly, the traditional ways of life of peoples inhabiting forests has come to be recognized again as being valuable. Such kinds of human activities could positively contribute to biodiversity, which is a future subject to be examined.

Macaques are a symbol of the forest environment that promises rich natural resources. Thus, the preservation of macaque populations contributes not only to the preservation of wildlife and the environment but also to our own quality of life.

PROPOSALS FOR MACAQUE PRESERVATION

I propose that it is essential to preserve macaque populations as much as possible. Although some of them are living under poor conditions, they represent a part of specific diversity. Firstly, we must determine macaque distribution and evaluate variability within each species. Secondly, we must take measures to preserve local populations in accordance with their optimum conditions of life. The first priority of preservation is almost always given to such superior ecosystems as those in National Parks or Conservation Areas from which human inhabitants are often excluded. However, preservation efforts should not stop at the establishment of these areas, even if they do carry a sufficient capacity of individuals to maintain genetic diversity in the future. The diversity in those populations cannot represent that of entire species. We should also preserve isolated, smallish, and sometimes commensal populations. National parks or Conservation areas tend to be established in the greater scale of mountainous areas, for example, those in the Western region of Thailand, including the Dawna Range. However, a number of macaque populations are found in isolated forests or mountains close to or inside towns or cities. They too may carry biological characters unique to their geographical locations, and thus, they are not insignificant populations in Northeastern, Eastern, and Central regions of Thailand, especially those rhesus populations deployed in the northeastern region.

Special management is required to preserve these commensal populations, which includes both the amelioration of their habitats and population and genetic control. As these populations are more or less isolated from other populations, genetic heterogeneity within each of them would soon be lost by inbreeding and genetic drift. Animal introduction from other populations or artificial insemination must be appropriately carried out. If animals or the semen of animals could be taken from troops located geographically closest to the target troop, there would be gene flow between the two troops that would presumably otherwise occur under natural conditions. Genetic monitoring on a regular basis is, of course, a prerequisite for this kind of genetic management.

Artificial hybridization and the introduction of conspecifics of unknown origin, on the other hand, should be strictly avoided. On our survey trips, we found that released pet macaques had managed to reproduce. This kind of "genetic pollution" spoils the specific gene pool, which must be regarded as an impediment to the entity of the species. Further, the transfer of a given troop to another habitat should be made carefully to preserve the original geographical variability.

Finally, ecological education is indispensable in the construction of better relations between humans and macaques and to preserve macaque biodiversity as much as possible.

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HERV Gene Information in Primates

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ABSTRACT.– Most of the endogenous retroviral genes integrated into the primate genome after the split of New World monkeys in the Oligocene era, approximately 33 million years ago. Because they can change the structure of adjacent genes and move between and within chromosomes they may play important roles in evolution as well as in many kinds of disease and the creation of genetic polymorphism. Comparative analysis of HERVs (human endogenous retroviruses) and their LTR (long terminal repeat) elements in the primate genomes will help us to understand the possible impact of HERV elements in the evolution and phylogeny of primates. For example, HERV-K LTR and SINE-R elements have been identified that have been subject to recent change in the course of primate evolution. They are specific elements to the human genome and could be related to biological function. The HERV-M element is related to the superfamily of HERV-K and is integrated into the periphilin gene as the truncated form, 5'LTR-*gag-pol*-3'LTR. PCR and RT-PCR approaches were conducted to investigate its evolutionary traces, indicating that the insertion of various retrotransposable elements in a common ancestor genome may make different transcript variants in different primate species. Examination of the HERV-W element revealed *env* fragments were detected on human chromosomes 1, 3-7, 12, 14, 17, 20, and X, whilst the *pol* fragments were detected on human chromosomes 2-8, 10-15, 20, 21, X, and Y. Bioinformatic blast search showed that almost full-length of the HERV-W family was identified on human chromosomes 1-8, 11-15, 17, 18, 21, and X. Expression analysis of HERV-W genes (*gag*, *pol*, and *env*) in human tissues by RT-PCR indicated that *gag* and *pol* were expressed in specific tissues whilst *env* was constituitively expressed in all tissues examined. DNA sequence based phylogenetic analysis indicated that the *gag*, *pol* and *env* genes have evolved independently during primate evolution. It will thus be of considerable interest to expand the current HERV (and other) gene information of various primates. The international network system greatly facilitates such studies in allowing us to understand Asian primates and thus should be actively supported with encouragement for further innovative research projects.

KEY WORDS: HERV elements, Gene information, Phylogeny, Evolution, Primates

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INTRODUCTION

Human endogenous retroviruses (HERVs) and long terminal repeat (LTR) -like elements are dispersed over 8% of the whole human genome (International Human Genome Sequencing Consortium, 2001). There are at least 22 distinct HERV families within the human genome (Tristem, 2000). HERVs originate by germ-cell infection of the exogenous retrovirus during primate evolution (Lower et al., 1996). Full length retroviral sequences may interact with cellular oncogenes (Varmus, 1982) and retroviral LTR elements have the capacity to exert a regulatory influence as promoters and enhancers of cellular genes (Akopov et al., 1998; Baust et al., 2000). Elucidation of the HERV elements should provide information about fundamental cellular activities and the pathogenesis of multifactorial diseases such as cancer and autoimmune disease (Nakagawa et al., 1997; Mayer, 2001).

The HERV-K family of human endogenous retroviral sequences was originally cloned from Syrian hamster intra-cisternal A type particles (Ono et al., 1986), has homology to mouse mammary tumour virus, and includes sequences that are expressed in normal placenta and leukemic cells (Simon et al., 1994). HERV-K elements probably entered the primate genome after the split of New World monkeys in the Oligocene era, 33-40 million years ago (Steinhuber et al., 1995). It is estimated that there are now approximately 25,000 copies of HERV-K LTRs in the human genome, and they have randomly transposed across the chromosomes in the course of human evolution (Leib-Mösch et al., 1993). The possibility that some of this increase has occurred recently was suggested by the presence of a functional integrase in HERV-K sequences (Kitamura et al., 1996). The ratio of synonymous to nonsynonymous substitutions suggests that evolutionary selection has recently been or is still operating (Zsiros et al., 1998). Medstrand and Mager (1998) have demonstrated that some clusters of HERV-K LTRs show low (average 1.5%) divergence; in comparisons across primate species, members of their cluster 9 are specific to the *Homo sapiens*. HERV-K LTRs bind host cell nuclear proteins and have the potential to activate neighboring genes (Akopov et al., 1998). A possible role of HERV-K elements in disease has been considered in relation to insulin dependent diabetes mellitus (Conrad et al., 1997), seminoma (Sauter et al., 1995) and HERV-K-T47D mammary carcinoma (Seifarth et al., 1998). Recently, HERV-M (HERV-K22), the oldest beta retrovirus, was identified in the human genome. The HERV-M proviral sequences were detected in hominoids, Old and New World monkeys, but not in prosimians. Compared to the other HERV-K families, most of their structural genes (*gag* and *env)* were severely truncated (Lavie et al., 2004).

SINE-R retroposons are derived from the endogenous retrovirus HERV-K family. SINE-R11, 14, 19 were isolated by colony blot hybridization using the 3′ long terminal repeat and small upstream flanking regions of HERV-K10 as probe (Ono et al., 1987). These elements entered the genome of hominoid primates after the split of Old World monkeys in the Oligocene period (Kim et al., 1999a,c,d). The SINE-R.C2 element was discovered in relation to a variable number of tandem repeat (VNTR) sequence within the third intron of the gene for C2, the second component of complement, located in class III of the major histocompatibility complex on the short arm of human chromosome 6 (Carroll et al., 1984; Zhu et al., 1992). By Southern blot analysis, SINE-R.C2 has been found to be confined to *Homo sapiens* (Zhu et al., 1994). Within the Xq21.3 block of homology to Yp11.2 that was created in the course of hominid evolution (Laval et al., 1998), two retroposons (HS307 and HS408) with 92 to 96% homology to SINE-R.C2 were reported (Kim et al., 1999c). SINE elements have generally arisen either from 7SL RNA or from tRNA, have been amplified many times in the genome, and have become fixed at certain stages of evolution. They share three prime sequences with LINE elements, which probably have an earlier evolutionary origin, and upon which they may depend for the reverse transcriptase activity necessary for retroposition (Okada et al., 1997).

A new HERV-W family has been identified by successive overlapping cDNA clones from multiple sclerosis virion (Komurian-Pradel et al., 1999). The HERV-W has been one of the most widespread investigated HERV elements, since the isolation of a HERV-W-related retrovirus (MSRV) from retroviral particles produced by cell cultures from patients with multiple sclerosis (Perron et al., 1997). The 7q21-22 region of human chromosome 7 has been identified as potentially involved in genetic susceptibility to multiple sclerosis (Charmley et al., 1991), contains a RGH proviral copy (HERV-H/RGH) and a complete HERV-W proviral copy (HERV-W/7q). The *env* gene of HERV-W/7q encods a protein expressed in fetal tissues and in placenta preferentially (Alliel et al., 1998). Recently, the HERV-W *env* protein, syncytin, was found to be involved in placental morphogenesis (Mi et al., 2000). The major sites of syncytin expression were placental syncytiotrophoblasts and multinucleated cells that originated from fetal trophoblasts. Here I review gene information of the retrotransposable elements, HERV-K, HERV-M, SINE-R, and HERV-W, in humans and primates.

GENOMIC FEATURES OF HERV-K LTR AND SINE-R ELEMENTS AND THE IMPLICATIONS FOR HUMAN DISEASES

The long terminal repeat (LTR) elements of the human endogenous retrovirus HERV-K have been found to be present in hominoids and Old World monkeys (Zhu et al., 1994), whilst HERV-K LTR-like elements were identified in New World monkeys (Kim et al., 1999a,e). In the human genome, several thousand copies of HERV-K LTR elements are found with an essentially random distribution. Some of the LTR elements have already been identified using human monochromosome and genomic DNAs (Kim and Crow, 1999; Kim et al., 2000), whilst additional human-specific HERV-K LTR elements were identified from the GenBank database (Medstrand and Mager, 1998) (AC002350, AC002400, AC002508, etc.). All of these LTR elements and the new HERV-K LTR elements that were transcribed in human brain tissue were used to construct a phylogenetic tree (Fig. 1). The HERV-K LTR elements were mainly divided into two groups through evolutionary divergence, although note that this is only weakly supported. The human-specific HERV-K LTR element (AL034407) has proliferated within human chromosome region Xq26 recently (Kim et al., 2000). Similarly, other HERV-K LTR elements (AC002350, AC002400, AC002508, L47334, U47924, Z80898) are only detected in the human genome (Medstand and Mager, 1998). Some HERV-K LTR elements (HKL-B7, HKL-B8, HKL-B10), identified in human brain cDNA, were closely related to human-specific HERV-K LTR elements, indicating that they may be related to brain function or neuropsychiatric disease.

SINE-R retroposons, derived from the HERV-K LTR elements (Ono et al., 1987; Zhu et al., 1992, 1994), have been found to be hominoid-specific by PCR analysis (Kim et al., 1999a,c). By BLAST search, cDNA prepared from the brain of a patient with schizophrenia (GenBank, accession no. AA772777) was found to have sequences containing a high degree of homology with the SINE-R11, 14, 19 (Ono et al., 1987), and with SINE-R.C2 (Zhu et al., 1994). HS307 and HS408 retroposons are located in Xq21.3 region of the human genome, transposed to the Yp11 after the separation of the chimpanzee and human lineages (Lambson et al., 1992). Both HS307 and HS408 retroposons show a high degree of sequence similarity with the schizophrenic brain cDNA derived from the postmortem brain tissue from the frontal cortex of an individual suffering from schizophrenia who committed suicide at the age of 34 years (Yee et al., 1998). Interestingly, Laval et al. (1998) showed evidence of linkage of both psychosis and degree of handedness (an index of cerebral

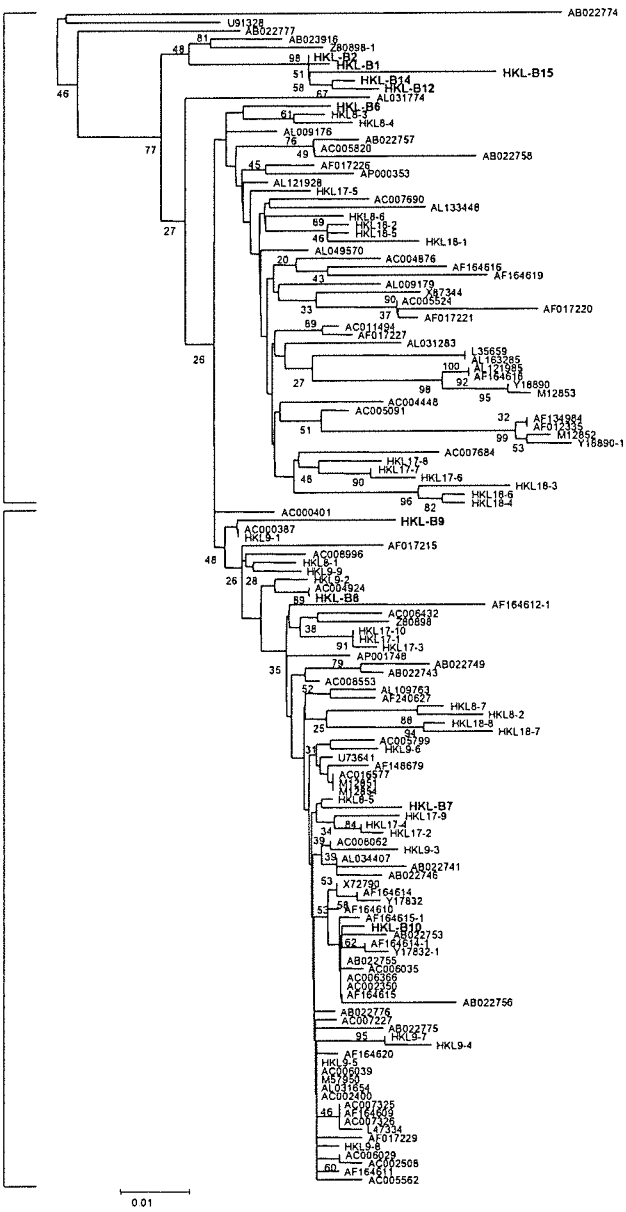

FIGURE 1. Phylogenetic tree obtained by neighbor-joining method for the HERV-K LTR family. Branch lengths are proportional to the distances between the taxa. The values at branch-points indicate the percentage support for a particular node after 100 bootstrap replications. The accession numbers of HERV-K LTR elements were obtained from the GenBank database. The HERV-K LTR elements (HKL-B1, HKL-B2, HKL-B6, HKL-B7, HKL-B8, HKL-B9, HKL-B10, HKL-B12, HKL-B14, HKL-B15) presented as bold letters were derived from human brain cDNA library.

asymmetry) to Xq21.3 region. Therefore, in order to explore the retroviral elements in Xq21.3 region, a YAC clone panel derived from the Xq21.3 region was examined and four HERV-K LTR elements found and characterized (Kim and Crow, 2001). Two of those elements (K-X10-5 and K-X13-1) have a high degree of sequence similarity to the human specific HERV-K LTRs. Elements of the SINE-R.C2 class have similarities to the clusters of the HERV-K LTR family (Fig. 2). SINE-R retroposons have a polypurine tract that serves as a primer binding site for plus-strand DNA synthesis from retrovirally mediated reverse transcription (Ono et al., 1987). In addition, they have a hormone responsive element and enhancer core of the HERV-K LTR element although the promoter is deleted. These SINE-R retroposons and HERV-K LTR elements could contribute to the structural change or genetic variation associated with neuropsychiatric disease and primate evolution. Akopov et al. (1998) noted that HERV-K sequences have the capacity to modify the expression of neighboring genes, and suggested that such modifications may have been acquired in the course of human evolution. The HERV-K-T47D-related LTR element mediates polyadenylation of cellular transcripts (Baust et al., 2000). Two HERV-K LTR elements have been detected in the human histocompatibility complex locus HLA-DQ (Kambhu et al., 1990). One LTR element (DQ-LTR3) of the HERV-K family at the HLA-DQB1 locus has been associated with rheumatoid arthritis (Seidl et al., 1999). An element described as almost identical to SINE-R.C2 derived from the HERV-K LTR element is the likely cause of Fukuyama-type muscular dystrophy (Kobayashi et al., 1998). Clearly, these HERV-K LTR and SINE-R elements deserve further investigation as potential leads to various human diseases.

IDENTIFICATION AND CHARACTERIZATION OF HERV-M AND PPHLN1 HYBRID TRANSCRIPTS

The HERV superfamily is one of the well-known repeat elements in the human genome, and the various families were phylogenetically characterized after completion of the human genome project (Tristem, 2000). The HERV-M family (HERV-K22) was recently identified and the genomic structure, localization, and phylogenetic relationship of HERV-M elements are reviewed elsewhere (Lavie et al., 2004). However, the HERV-M located within the *PPHLN1* gene has a disrupted open reading frame yet seems to overcome the situation of functionlessness in the human genome with a gene-like transcription strategy during primate evolution. Generally, HERV families do not use the donor and acceptor sites like a eukaryotic cell, their PBS (promoter binding site) and polyadenylation signals are used during their transcription. Phylogenetic analysis suggests that more than 45 million years was sufficient for changing of the HERV-M from a viral to cellular gene with the accumulation of mutations.

The HERV-M could make various hybrid transcripts for the *PPHLN1* gene in coding regions with an alternative splicing pattern like a normal eukaryotic gene transcript by data base screening (EST and Refseq mRNA data base) with a HERV-M consensus sequence. The HERV-M sequence was expressed in human brain, prostate, testis, heart, kidney, liver, lung, placenta, skeletal muscle, spleen, thymus, and uterus, but not in kidney, liver orlung. However, RT-PCR amplification suggested it was not detected in as many of the tested tissues in the crab-eating and squirrel monkey being absent in testis, kidney, liver, lung, and spleen (Huh et al., 2005).

Insertion and expression events of the HERV-M elements seem to occur independently. Although the insertion of HERV-M happened before the divergence of New and Old World monkeys, expression of HERV-M in the *PPHLN1* gene has been found only in humans and crab-eating and squirrel monkeys. After the integration of HERV-M, a series of repeat element insertions may affect the instability of the primate genome, and subsequently the gene transcription mechanisms. Although the exact mechanism of the *PPHLN1* gene transcription related to HERV-M is not yet clear, elucidation of such events are likely to provide be a good research model of HERV exonization.

The *PPHLN1* gene harbors different repeat elements of HERV-M, *Alu*sq, and L1ME4a within the human chromosome 12q12 region.

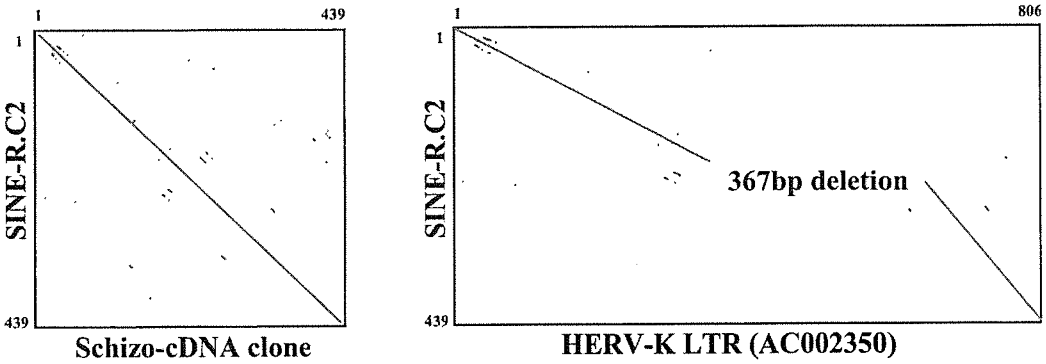

FIGURE 2. Dot plot matrix of SINE-R retroposon relative to sequences from the schizophrenic brain cDNA (schizo-cDNA) clone and HERV-K LTR element (GenBank, accession no. AC002350).

The insertion event of HERV-M occurred in New World monkeys approximately 50 million years ago, and *Alu*sq and L1ME4a were inserted into the mammalian genome before the divergence of simian and prosimian lineages about 60-70 million years ago. This allows us to speculate that lineage specific duplication events could have occurred independently.

BIOINFORMATIC ANALYSIS AND MOLECULAR

The HERV-W family has been one of the most broadly investigated HERVs during the last few years. It consists of an estimated 30 to 100 provirus copies per haploid human genome and is phylogenetically related to ERV-9 and RTLV-H families. HERV-W *gag* and *pol* open reading frames (ORFs) of the family have acquired frameshifts and stop codons by deletions/ insertions, whereas a complete *env* ORF encoding an envelope was found on the cl.PH74 placental cDNA clone and on the genomic RG083M05 clone (Blond et al., 1999). HERV-W may have a chimeric genome structure since the murine type C and simian type D retroviruses show strong homology with the *pol* and *env* genes, respectively. The HERV-W on chromosome 7 has 100% identity to syncytin cDNA, which is expressed specifically in the syncytiotrophoblast layer of the placenta (Alliel et al., 1998; Mi et al., 2000). This observation implicates the potential HERV-W family involvment in human placental development. That the product of the HERV-W *env* gene is a highly fusogenic membrane glycoprotein that induces the formation of syncytia on interaction with the type D mammalian retrovirus receptor (Blond et al., 2000) supports this notion as well as suggesting a direct role in human trophoblast cell fusion and differentiation (Frendo et al., 2003). Recently, the HERV-W gene product was shown to block infection by exogenous retroviruses, suggesting that expressed HERV genes could have beneficial functions to the host (Ponferrada et al., 2003). The possibility that they have relevance to pathogenic capacity could be subjected to change in primate evolution and could be associated with variation between different tissues and gene expression.

The HERV-W family are related to multiple sclerosis-associated retrovirus (MSRV) sequences identified in particles recovered from monocyte cultures from patients with multiple sclerosis. Retroviral sequences belonging to HERV-W family were also identified in brain of individual with schizophrenia, increasing the possibility that HERV-W family could be involved in human brain disease (Karlsson et al., 2001). Such HERV-W *env* families are located on human chromosomes 1, 3, 4, 5, 6, 7, 12, 14, 17, 20, and X (Kim and Lee, 2001), while HERV-W *pol* families are located on human chromsomes 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14, 15, 20, 21, X, and Y (Kim, 2001).

Within the HERV-W family, *env* and *pol* genes showed a high degree of nucleotide sequence similarity (91.6-99.6%), but translation of these sequences revealed the presence of both frameshifts and premature termination codons caused by deletion/insertion or point mutations in some clones (Kim et al., 1999b). Outside of humans, the *pol* and *env* gene sequences of the HERV-W family were detected by PCR in hominoids and Old World monkeys, but not in New World monkeys. The HERV-W family has been phylogenetically analyzed from the databases and divided into three different subfamilies of the human genome derived HERV-W group (Costas, 2002). The average divergence between sequences for each of the subfamilies indicated that most of the HERV-W elements were inserted within the genome during a short period of evolutionary time.

To date, BLAST search of the DDBJ/EMBL/GenBank databases for HERV-W family reveals 140 sequences representing 39 HERV-W proviruses, 40 full-length HERV-W retroposons, and 61 truncated HERV-W retrosequences (Costas, 2002). The number of identified HERV-W related fragments comprises of at least 70 copies for *gag* and 30 copies for *env* per haploid genome, and is correlated with the increase in complexity from *env* to *gag* and *pro* regions as previously described (Voisset et al., 2000). From the 15 HERV-W *env* groups found within the human genome (Kim and Lee, 2001),one of them, clone W-7-1 from chromosome 7, showed identical sequences with those of brain, testis, kidney, lung, placenta, skeletal muscle, and uterus of normal tissues (WE1-5, WE3-8, WE5-4, WE7-2, WE8-1, WE9-3, WE10-14), suggesting that they are actively expressed in various normal tissues (Fig. 3) (Yi et al., 2004). Other HERV families are expressed in normal tissues and cancer cells. For example, HERV-E family on human chromosome 17q11 was expressed specifically in human pancreas and thyroid gland with two major transcripts, 3.3 and 4.1kb in size (Shiroma et al., 2001). HERV-H *env* transcripts have been detected in both T-cell leukemia cell lines and lymphocytes from healthy blood donors by RT-PCR (Lindeskog and Blomberg, 1997). Recently, the tissue-specific expression of two HERVs transcripts (HERV-K *cORF/rec* and HERV-R *env*) was found in normal human tissues (kidney, tongue, heart, liver, central nervous system) during embryogenesis, suggesting a possible role in the development and differentiation of human tissues (Andersson et al., 2002). In a comparative analysis between normal and cancer cells, the HERV-W *gag* gene was expressed in normal brain tissue, whereas no expression but not in PFSK-1 cancer cells derived from brain tissue. The HERV-W *pol* gene was also expressed in prostate tissue, but not in PC3 cancer cells derived from prostate tissue (Fig. 3). In the case of the HERV-E *env* gene, it was actively expressed in prostate carcinoma tissues and cell lines, but not in normal prostate tissues and cells as detected by RT-PCR, RNA *in situ* hybridization, and Northern blot hybridization (Wang-Johanning et al., 2003). No expression of the HERV-E *env* gene was detected in breast tissues, but in contrast, HERV-K *env* transcripts were detected in most breast cancer cell lines and many breast tumor tissues (Wang-Johanning et al., 2002). Such HERV-K *gag* transcripts were found in gonocytes of all genadoblastomas and in neoplastic germ cell tumours whilst the transcriptional activity of the HERV-K *gag* gene was 5- to 10-fold higher in blood cells of leukemia patients than in normal cells (Depil et al., 2002).

Whilst products of the HERV-W *env* gene are implicated in a direct role in human trophoblast cell fusion and differentiation (Frendo et al., 2003) and, via blocking the infection by an exogenous retrovirus, may have beneficial function to the host (Ponferrada, 2003), the relationship of HERV elements in general and human diseases remains contraversial. Reports of detection of various HERV mRNA, HERV proteins, and even HERV particles in human diseases, especially cancer, are becoming numerous, but remain correlative. The HERV-W *env* gene sequences have been characterized in some human cancer

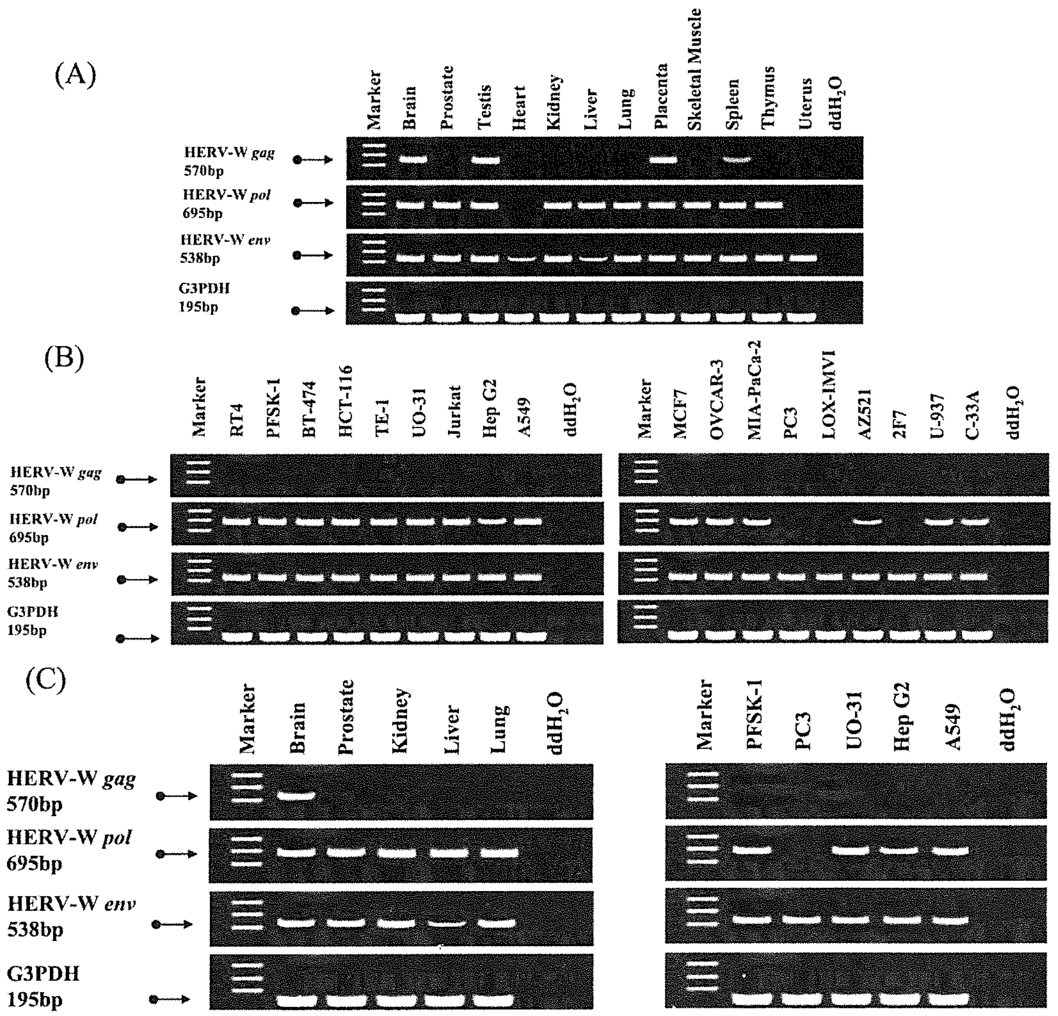

FIGURE 3. RT-PCR analysis of mRNA for the expression of HERV-W genes (*gag, pol,* and *env*) from various (A) human tissues and (B) cancer cells. (C) Comparative expression pattern between human normal tissues and cancer cells. As a standard control, G3PDH was amplified by the primers GPH-S (5′-CAAAGTTGTCATGGATGACC-3′, bases 31721-31740) and GPH-AS (5′-CCATGGAGAAGGCTGGGG-3′, bases 31898-31915) from the human G3PDH gene (GenBank accession no. AC068657). PCR amplification without RT reaction with pure mRNA samples was performed, indicating that the prepared mRNA samples from tissues and cells did not contain genomic DNA (data not shown).

cell lines to examine the role of HERV in human cancer (Yi et al., 2002) and to investigate the comparative analysis of HERV-W families between humans and monkeys to understand their tissue distribution, evolution, and phylogenetic relationships (Yi et al., 2003). In bioinformatic analysis, a BLASTN query on the EST (expressed sequence tag) database with HERV-W showed multiple transcripts in various human tissues such as bladder, brain, breast, colon, hypothalamus, kidney, liver, lung, ovary, parathyroid, placenta, prostate, skin, stomach, testis, and uterus (data not shown), supporting that HERV-W families could have a biological role in multiple human tissues. Taken together, these data suggest that some recently proliferated and expressed HERV-W families are active in the genome of tissues and cancer cells, and that they therefore could have a functional role in human tissues and cancer cells.

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Primate Evolution in the Neogene Southeast Asia: Recent Discoveries of Hominoid Fossils in Thailand

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ABSTRACT.– **Little is known about primate evolution in the Neogene Southeast Asia, but some Miocene hominoid fossils have recently been discovered from Thailand. These findings are beginning to shed light on the study of Neogene primates in this region.**

KEY WORDS: Neogene, Miocene, Hominoid, Southeast Asia, Thailand

INTRODUCTION

The Neogene is the last part of the Tertiary Period, including the Miocene (23.5 - 5 MYA) and Pliocene (5 - 1.8 MYA). This period is quite important and interesting for anthropologists and primatologists, because it is during the Neogene that extant hominoids and cercopithecoids were diversified into various lineages. The divergence between these two modern superfamilies probably occurred in Africa during the Early Oligocene (~30 MYA), but there is still little fossil evidence from that time. In eastern Eurasia, primitive catarrhines (Pliopithecidae) appeared in the fossil record in the mid-Early Miocene (17-18 MYA). They are considered to be a surviving lineage of primitive catarrhines that originated in Africa prior to the divergence between the cercopithecoids and hominoids, although their ancestors have yet to be discovered in Africa. In Southeast Asian countries, however, there is no pliopithecid fossil except for a single isolated lower molar from Ban San Klang in northern Thailand (Suteethorn et al., 1990), which could belong to the genus *Dionysopithecus* and tentatively classified as *D. orientalis* (Harrison and Gu, 1999). Miocene large-bodied hominoids have been recovered from Siwaliks and China for decades, and some hominoid fossils have been reported recently from Thailand (Chaimanee et al., 2003, 2004; Kunimatsu et al., 2000, 2002). These are discussed in detail later. Cercopithecoids are latecomers in eastern Eurasia. The earliest cercopithecoid in eastern Eurasia appeared in the Siwaliks in the late Late Miocene (~6-7 MYA), being coincident with the hominoid decline in the fossil record of this area. A few cercopithecoid teeth have been known from the latest Miocene of eastern China (~5 MYA). The large cercopithecine *Procynocephalus* have

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been discovered in the Plio-Pleistocene deposits in the Siwaliks and China. It is, however, in the Pleistocene that cercopithecoids appeared in the Southeast Asian fossil record, and there is no hard evidence for discussing their evolution during the Neogene in this region. In this article, we review the present situation of the hominoid evolutionary study in Southeast Asia.

LARGE-BODIED HOMINOIDS

Primitive hominoids flourished in the Afro-Arabian landmass during the Early Miocene, and in the late Early Miocene (16-17 MYA) large-bodied hominoids migrated from Africa into western Eurasia. In eastern Eurasia, however, hominoids appeared in the fossil record a few million years later. Until recent, Miocene hominoids in eastern Eurasia have been discovered mainly from two regions: Siwaliks in the northwestern part of the Indian subcontinent and Yunnan Province in China. The oldest occurrence of *Sivapithecus* in the Siwaliks, is dated at ~12.5 MYA. The oldest hominoid site in Yunnan is Kaiyuan (Xiaolongtan). Its age is estimated as ~10 MYA or slightly older (Pickford and Liu, 2001; Pickford et al., 2004). Both of these regions are located north of the Tropic of Cancer. In southern areas like the Indochina peninsula and Southeast Asian islands, little is known about the hominoid evolution in the Neogene, but recent findings of large-bodied hominoids in Thailand (Chiang Muan, Phayao Province & Tha Chang, Nakhon Ratchasima Province (= Khorat)) have started shedding new light on this matter.

CHIANG MUAN HOMINOID

Chiang Muan Basin is one of the intermontane basins in northern Thailand. In this basin, the Chiang Muan Mine Company (CMMC) has been excavating lignite at an opencast mine. At the locality of mine CMu6 an isolated upper molar of a large-bodied hominoid was discovered in January 2000. This finding was immediately reported to the mine office, and it was soon announced at the 16th annual meeting of the Primatological Society of Japan (Kunimatsu et al., 2000). This specimen (CMu6-1'00) was derived from the Lower Lignite unit of the Chiang Muan Formation (Nagaoka and Suganuma, 2002), and was described by Kunimatsu et al. (2004). In December of 2001, an additional hominoid specimen (CMu15-5'01), a mesial fragment of a lower molar, was discovered by the Thai-Japanese Paleontological Expedition Team (TJPET) from a different horizon corresponding to the Upper Lignite unit, which was several tens of meters above the Lower Lignite unit (Kunimatsu et al., 2002, 2005b). Chaimanee et al. (2003) reported more isolated teeth of a large-bodied hominoid, which were derived from the Upper Lignite unit, and they tentatively referred them to the genus *Lufengpithecus* with a new specific name, that is, ? *Lufengpithecus chiangmuanensis*.

In December 2003, a small fragment of a molar (CMu9-1'03) was collected from the Upper Lignite unit by TJPET. This specimen has not yet been described elsewhere so we provide its description here. The fragmentary nature of the specimen makes its precise assignment difficult, but it is most likely a right upper M3 of a large-bodied hominoid, which preserves the enamel but has lost the dentine (Fig. 1). The buccal part of the paracone is largely missing, and the cervical part of the metacone and hypocone is broken off. The remaining part of the crown is ~8 mm both mesiodistally and buccolingually, and the complete size may have been ~9 x ~9 mm (very rough estimate). The protocone is low and voluminous, and it is apparently the largest cusp. The paracone would be the next largest if it were complete. The metacone is considerably smaller compared to the mesial cusps, and the hypocone is strongly reduced to be a tiny tubercle on the distal margin of the crown. The protocone has three crests. The mesiobuccal crest is short and ends at a moderately developed protoconule. The distobuccal crest is thick and touches the mesiolingual crest from the metacone apex to form the crista obliqua.

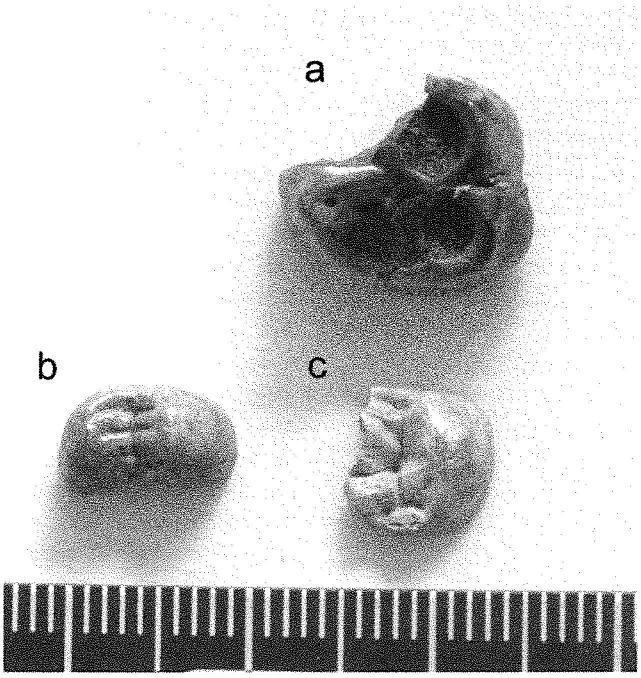

FIGURE 1. Large-bodied hominoid specimens discovered from Chiang Muan by the Thai-Japanese Paleontological Expedition Team. a) CMu6-1'00, a right upper M1 or M2; b) CMu15-05'01, a mesial fragment of a right lower molar; c) CMu9-1'03, a right upper M3.

The distal crest comes out from near the protocone apex and runs down distally and slightly buccally towards the tiny tubercle that corresponds to the hypocone. On the mesiolingual aspect of the protocone remains a faint trace of the lingual cingulum. A part of the mesial fovea is preserved. The occlusal grooves are markedly incised and clearly define the cusp boundaries.

The hominoid molars collected from the Upper Lignite unit are similar in size to those of extant orangutans (*Pongo pygmaeus*), but they lack the extensive fine crenulation on the occlusal surfaces, which is characteristic to the latter. A relatively broad lower incisor (Chaimanee et al., 2003) might be a feature that suggests some phyletic relationship of *L. chiangmuanensis* to extant orangutans which, together with chimpanzees, have a relatively broad lower incisor region compared to other anthropoid primates (Pickford, 2005).

Another important point is the relationship of *L. chiangmuanensis* to the *Lufengpithecus* species from Yunnan Province in China, especially to the hominoid material known from Kaiyuan, which was originally named *Dryopithecus keiyuanensis* (Woo and Chow, 1957), and is often included in the genus *Lufengpithecus* by recent researchers (Harrison et al., 2002). Kaiyuan is geographically close to Chiang Muan, being located only several hundred kilometers north of the latter. The mammalian faunas of these two sites suggest that they are chronologically close to each other as well (Pickford et al., 2004). Pickford and Liu (2001) estimated the age of Kaiyuan to be ~10 MYA, but it could be slightly older (Pickford et al., 2004). A preliminary paleomagnetostratigraphic study has suggested that the age of the Chiang Muan hominoid is 10-12 MYA, around the boundary of the Middle/Late Miocene (Suganuma et al., 2002).

A more detailed paleomagnetostratigraphic study is going on in order to determine the age of Chiang Muan hominoid more precisely (Suganuma pers. comm.).

Chronological and geographical similarities themselves do not immediately indicate the phylogenetic closeness of fossil materials. Nonetheless, these similarities necessitate a careful treatment of the two hominoid materials from Kaiyuan and Chiang Muan (see Kunimatsu et al., 2004, 2005a, 2005b and Pickford et al., 2004 for more detailed discussions). Despite of the ambiguities caused by the poor nature of the hominoid material, Chiang Muan is quite an important fossil site for paleoprimatology, as it may provide information on the earliest stage of the hominoid dispersal into Southeast Asia.

KHORAT HOMINOID

A well-preserved mandible of a large-bodied hominoid was recently reported from Nakhon Ratchasima Province (or Khorat), northeastern Thailand, and was assigned to *Khoratpithecus piriyai* gen. et sp. nov. by Chaimanee et al. (2004). The type mandible is the only specimen of *K. piriyai* ever known to date. It is registered in the collection of Rajabhat Institute of Nakhon Ratchasima (RIN), with the accession number of RIN765. Chaimanee et al. (2004) have suggested that *K. piriyai* is closely related to extant orangutans. The most important feature is the lack of the digastric fossa on the inferior surface of the symphysis. This feature suggests that the anterior belly of the digastric muscle may have been missing as in the extant orangutans (Chaimanee et al., 2004). The problem is, however, whether the absence of evidence can be or cannot be the evidence of absence. The shape and degree of impression of the digastric fossa are various in living and fossil hominoids. At present, it can be said that *K. piriyai* is an interesting taxon regarding to the origins of extant orangutans, but the hard evidence is still too little to make satisfactory perspectives for their evolution.

RIN765 is said to have originated from a sand pit in Tha Chang, Chalerm Phrakiat District, Nakhon Ratchasima Province. In this area, there are several sandpits along the Mun River. Fossils have been yielded from these sand pits for years, though the majority of the sandpits have already been abandoned. In most cases, these fossils were discovered and collected through sand pit operation by local workers, who were not trained in either paleontology or geology. One of the authors (P. Jintasakul) has tried to rescue these fossils and has accumulated hundreds of them in the collection of Rajabhat Institute of Nakhon Ratchasima. Some other fossils have been brought to other public organizations of this area, such as the district office and a local library. It seems, however, that not a few fossils may have been lost from science before they became recognized by public organizations.

RIN765 came to the notice of the scientific community in Thailand around July of 2002. At that time, there were only two active sand pits in the area, which we have called Tha Chang Sand Pit 8 and 9 (TCSP8 & 9). The situation of discovery is not precisely known, but according to our interviews to local sandpit workers within a few months after July 2002, the hominoid mandible was discovered at TCSP8 (GPS Datum WGS84: N15° 01' 47.8" E102° 16' 33.5", Fig. 2) during sand excavation and was collected by local workers (probably around May 2002). Then, the specimen was handed to an amateur collector who eventually agreed to donate it to the Rajabhat Institute of Nakhon Ratchasima. As expected in such a situation, there is no reliable information about the horizon, from which RIN765 was derived. Some local workers told us that the hominoid mandible had been found several meters below the boundary between the upper and lower deposits. This sand pit (TCSP8) was then flooded through heavy rain in September/October 2002.

Recent studies of the mammalian fossils from these Tha Chang sandpits (Nakaya et al., 2003a; Saegusa et al., 2005) suggest that they include multiple faunal assemblages of different

FIGURE 2. Tha Chang Sandpit 8, from which the type mandible of Khoratpithecus piriyai is said to have been discovered. Photograph taken by Y. Kunimatsu in January 2002.

ages, that is, Middle Miocene, Late Miocene, and Pleistocene. Under the above-mentioned circumstances, the majority of the fossils lack the precise locality data so that determining the ages of these sandpit deposits is problematic. Based on the circumstantial evidence, however, each sandpit appears to contain only one of these faunal assemblages or two at most. As for the TCSP8, a well-preserved anthracothere skull was discovered and collected directly by the members of TJPET and RIN at this sandpit in February of 2002. This specimen is still under study, but preliminary observations suggest that it may belong to a primitive form of *Merycopotamus* (Nakaya et al., 2003b). Recently, Lihoreau et al. (2004) described a new species of this genus, *M. medioximus*, based on the material collected from the Nagri and lower Dhok Pathan Formations of the Siwaliks, and they considered it to be more derived than *M. pusillus* but more primitive than *M. dissimilis*. Although the affinity of the Tha Chang anthracothere has yet to be determined, it may belong to a similar grade with *M. medioximus*. Combined with the other mammalian fossils that are thought to have derived from TCSP8, the presence of this primitive *Merycopotamus* suggests that the age of this sandpit is most likely to be the Late Miocene (Saegusa et al., 2005). This is broadly a similar age to those of the *Lufengpithecus* sites of Yunnan Province (Kaiyuan (~10-11 MYA), Lufeng (~8 MYA) and Yuanmou (~9 MYA).

DISCUSSION

At present, the study of the Neogene primate evolution in Southeast Asian countries is just beginning with the discoveries of two fossil sites that have yielded large-bodied hominoids in Thailand. One of them (Chiang Muan) is interesting since further fieldwork at the site might shed new light on understanding the early stage of the hominoid dispersal into eastern Eurasia. As Chiang Muan and the oldest large-bodied homioid site in China (Kaiyuan) are opencast mines of lignite, which was formed during the Miocene, it would be also interesting to investigate other Miocene lignite mines in Thailand and in other Southeast Asian countries.

Khoratpithecus piriyai from Nakhon Ratchasima might be related to the ancestry of extant orangutans, but the present hypodigm consists of only one specimen whose chronological age can be estimated only vaguely to be Late Miocene. More specimens, of course, are needed, but it would be quite a difficult task to discover additional specimens as the hominoid-yielding sandpit is now submerged under the water. Nonetheless, since there are still some other active sandpits in the same region, it is possible that more hominoid fossils may be recovered if we keep paying attention to the ongoing operations at these sandpits.

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Establishment of the Primate Research Unit for the Promotion of Biomedical and Biodiversity Research in Thailand

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ABSTRACT.– **The Primate Research Unit, previously called the Primate Unit Cell, was established in 1970 by the late Professor Dr. M.R. Puttipongse Varavudhi. Long-tailed macaques (*Macaca fascicularis*) were chosen because they are a non-seasonal breeder. Approximately 100 individuals were introduced and successfully reared and bred. The research activities in the Primate Research Unit have changed every decade. Initially (1970-1980), the baseline levels of hormone patterns and reproductive functions in both sexes of captive monkeys were evaluated. In the next decade (1980-1990), the data and experience were used to examine the influence of contraceptive agents on fertility of long-tailed macaques. During 1990-2000, research subjects were elaborated to study the influences of drugs, such as methimazole, morphine, and ketamine hydrochloride, on changes in reproductive hormones. Currently (2001-date), the Primate Research Unit emphases two fields of research, firstly the reproductive physiology and secondly the biodiversity of macaque monkeys. As more than half of long-tailed macaques reared at the Primate Research Unit are now in their menopausal and andropausal stages, we plan to expand our research into aging in monkeys to take advantage of this rare and valuable resource. The biodiversity study on macaques will be expanded to other Southeast Asian countries in the near future with the Primate Research Unit functioning as the central hub of the currently established international network of SE Asian Primatologists.**

KEY WORDS: Primate Research Unit, *Macaca fascicularis*, Biodiversity, Reproductive physiology, Aging

BACKGROUND

Since the end of World War II, the population of humankind has rapidly increased causing various problems including the food shortage. Many national and international organizations called attention to those issues and financially supported the research on reproduction and contraception in many countries. To carry out physiological and pharmacological research, experimental animals are indispensable. The vast majority of animal research has used established experimental animals such as mice and rats, largely due to their ease to breed and maintain laboratory culture and their fast generation times. However, some scientific questions cannot be answered by using those rodent species. As the closest phylogenetic relative to humans, non-human primates play an indispensable role in biomedical research.

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At the middle of the twentieth century, capturing wild monkeys for research was restricted in Thailand. The late Dr. Puttipongse Varavudhi, Professor at the Department of Biology, Faculty of Science, Chulalongkorn University, realized the importance of non-human primates as a model system for biomedical research and established the breeding colony of long-tailed macaques (*Macaca fascicularis*). This, the first Primate laboratory in Thailand, was named the "Primate Unit Cell" in 1970, in the cooperation with the WHO Collaborating Center for Clinical Research, the Department of Anatomy and Physiology of California University, and the Department of Anatomy of Mahidol University. Approximately one-hundred individuals of long-tailed macaques were introduced, and successfully reared and bred. The reasons why long-tailed macaques were chosen are that they are non-seasonal breeders, breeding throughout the year (Tangpraprutgul and Varavudhi, 1982) and that they can be found throughout Thailand (Fooden, 1995).

The initial objectives of establishment of the "Primate Unit Cell" were 1) to establish facilities for maintaining the colony of long-tailed macaques, 2) to collect baseline physiological data in male and female monkeys, 3) to study the mechanism of pituitary gonadotropin release, 4) to understand the mechanism of implantation in pregnant female monkeys, and 5) to investigate the effects of post-coital contraceptive agents. During the first decade (1970-1980), profiles of hormones related to the reproductive system (estrogen, progesterone, testosterone, luteinizing hormone, prolactin and cortisol), were determined in immature, pubertal and adult male and female monkeys as well as in pregnant female monkeys. It was found that female long-tailed macaques showed a similar reproductive function and hormonal patterns with those of women. The menstrual cycle length is 30 days with 3-day bleeding (Varavudhi and Yodying-yuad, 1982; Varavudhi et al., 1982a,b; Varavudhi and Tangpraprutgul. 1983; Trisom-boon et al., 2004a). Average levels of testosterone begin to rise in pubertal male monkeys (3-4 years old), especially during sleep (Varavudhi et al., 1982a). Significant differences in serum testosterone levels between the day and night was observed in pubertal male monkeys, but disappeared in adults, the same trends as those observed in humans (Bremer et al., 1983).

After this extensive baseline data set was obtained, in the following decade (1980-1990), testing of contraceptive agents such as gossypol (a drug refined from cottonseed oil) and bromocryptine (dopamine agonist) were performed (Varavudhi et al., 1985). Then, during 1990-2000, research into the reproductive physiology of long-tailed macaques was elaborated to include the influences of drugs upon reproductive functions, through the change in hormones, such as methimazole (drug for hyperthyroidism treatment; Chongchareon et al., 1993), and ketamine hydrochloride (Malaivijitnond and Varavudhi, 1996; Malai-vijitnond et al., 1998). Long-tailed macaques were also chosen as an experimental animal to research the effects of narcotic drugs since this is encumbered by prevailing ethical and legal standards in humans. The knowledge attained was applied to clinical use in humans afterwards (Setheetham and Varavudhi, 1993; Malaivijitnond and Varavudhi, 1998), illustrating the biomedical research success and contribution of the Primate Research Unit.

In 1988, the "Primate Unit Cell", led by the late Prof. Puttipongse Varavudhi (Varavudhi et al., 1989), started field research on macaque monkeys in Thailand in collaboration with the Primate Research Institute, Kyoto University, led by the late Prof. Osamu Takenaka (Takenaka et al., 1989). The project was carried out for about five years with partial support from Chulalongkorn University.

CURRENT ACTIVITIES

Recently, the use of the indigenous herb, *Pueraria mirifica*, containing phytoestrogen, has become popular in Thailand as an

TABLE 1. The lists of laboratories and/or institutes where they have a collaborative research with the Primate Research Unit.

Region	Location	Subject
1. Chulalongkorn University	a. Malaria Research Unit, Faculty of Science	a. Effects of herbs on malaria in long-tailed macaques
	b. Malaria Laboratory, Faculty of Medicine	b. Detection of malaria among primate populations in Thailand
	c. Center of Excellence in Viral Hepatitis, Faculty of Medicine	c. Detection of viral hepatitis in macaque monkeys
2. National (Thailand)	a. Faculty of Science and Technology, Nakhon Sawan Ratchapat University	a. Hybridization and genetic variation of rhesus macaques at Loei Province
	b. Faculty of Science, Chiangmai University	b. Evolution and genetic variation of long-tailed macaques in Thailand
3. International	a. Primate Research Institute, Kyoto University	a. Biodiversity of macaque monkeys in SE Asia; and co-advisor of graduate students
	b. the Laboratory of Veterinary Physiology, Tokyo University of Agriculture and Technology	b. Effects of phytoestrogens on reproductive system in long-tailed macaques; and co-advisor of graduate students
	c. Department of Physiology, Nippon Medical School	c. Effect of phytoestrogens on breast cancer; and co-advisor of graduate students
	d. Laboratory of Zoology, Bogor Agricultural University, in Indonesia	d. Biodiversity of macaque monkeys in SE Asia
	e. Saigon Zoo-Botanical Garden, in Vietnam	e. Biodiversity of macaque monkeys in SE Asia
	f. Department of Biology, National University of Laos, in Lao P.D.R.	f. Biodiversity of macaque monkeys in SE Asia

alternative contraception medicine to estrogen in humans. Its actual effects on reproductive function and menstrual cycle therefore were required to be determined using model animals. Long-tailed macaques were again deemed to be adequate experimental animals because the indispensable controls on dietary and environment factors required for valid studies are ethically not possible in human subjects (Trisomboon et al., 2004a,b; 2005).

In the year 2001, the "Primate Unit Cell", led by Dr. Suchinda Malaivijitnond, succeeded to get support from Thailand Research Fund (TRF) to initiate research on the biodiversity of long-tailed macaques in Thailand (Malaivijitnond et al., 2004). In collaboration with the Primate Research Institute, led by Dr. Yuzuru Hamada, the biodiversity study on long-tailed macaques in Thailand has continued and been expanded to include four other macaque species. This project has also been approved and granted by the Japanese Society for the Promotion of Science (JSPS) (Malaivijitnond and Varavudhi, 2002; Malaivijitnod et al., 2005; Hamada et al., 2004; 2005a,b,c).

In 2003, Chulalongkorn University promoted the establishment of Research Unit, and approved the promotion of "Primate Unit Cell" to the "Primate Research Unit". Two fields of research have been emphasized; Reproductive physiology and Biodiversity of primates, mainly macaque monkeys. Thus, the current objectives of the "Primate Research Unit" have been slightly changed as follows:

1. To be the Center for medicinal herb testing on reproductive physiology and toxicity using mammals, especially long-tailed macaques, as models.
2. To be the Center of Excellence on the biodiversity study in primates, mainly macaque monkeys, in Thailand.
3. To establish the DNA bank of primates in Thailand.
4. To be the Center of information on primates in Thailand, serving both Thai and foreign researchers.

We are conducting collaborative research with many laboratories in Chulalongkorn

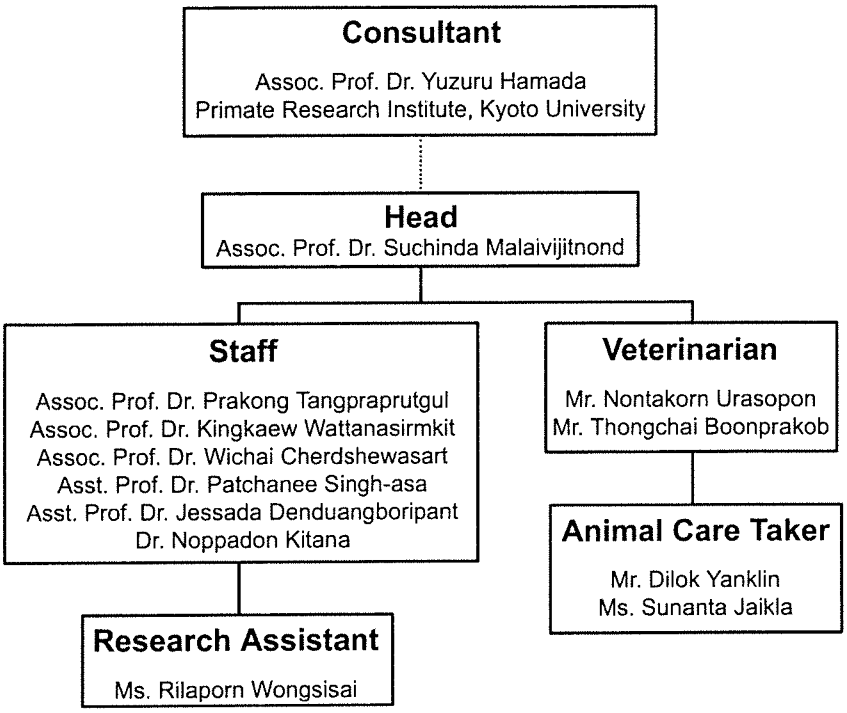

FIGURE 1. The current organization of the Primate Research Unit.

University and outsides as listed in Table 1. The research activities of the Primate Research Unit are supported by Chulalongkorn University, the Thailand Research Fund, the TRF/BIOTEC Special Program for Biodiversity Research and Training, Ministry of University Affair of Thailand, and National Research Council of Thailand. The current organization of the Primate Research Unit is shown in Fig.1.

PERSPECTIVES OF STUDIES IN THE PRIMATE RESEARCH UNIT

The Primate Research Unit will actively conduct research on primates, both based in the laboratory and in the field. There are 3 species of macaque monkeys in our laboratory at present; long-tailed macaques, assamese macaques (Macaca assamensis) and pig-tailed macaques (Macaca nemestrina) (Table 2). There are a total of 80 long-tailed macaques (53 females and 27 males) with more than half of them in their menopausal and andropausal stages, this provides a good opportunity for aging research including osteoporosis and atherosclerosis (Fig. 2). The production of aged monkeys is difficult, because it takes at least 20 years, highlighting the value of the Primate Research Unit. Aging research has also been especially helpful for understanding neurological phenomena like learning and memory deficits, visual impairment, neurochemical changes, and neuropathology.

TABLE 2. Number of macaque monkeys at Primate Research Unit

Species	Female	Male	Total
M. fascicularis	53	27	80
M. assamensis (Adult)	1	1	2
M. nemestrina (Adult)	1	1	2
Total	**55**	**29**	**84**

Thus, one of the main current researches in the laboratory will be the aging, using long-tailed macaques as a model. On the other hand,

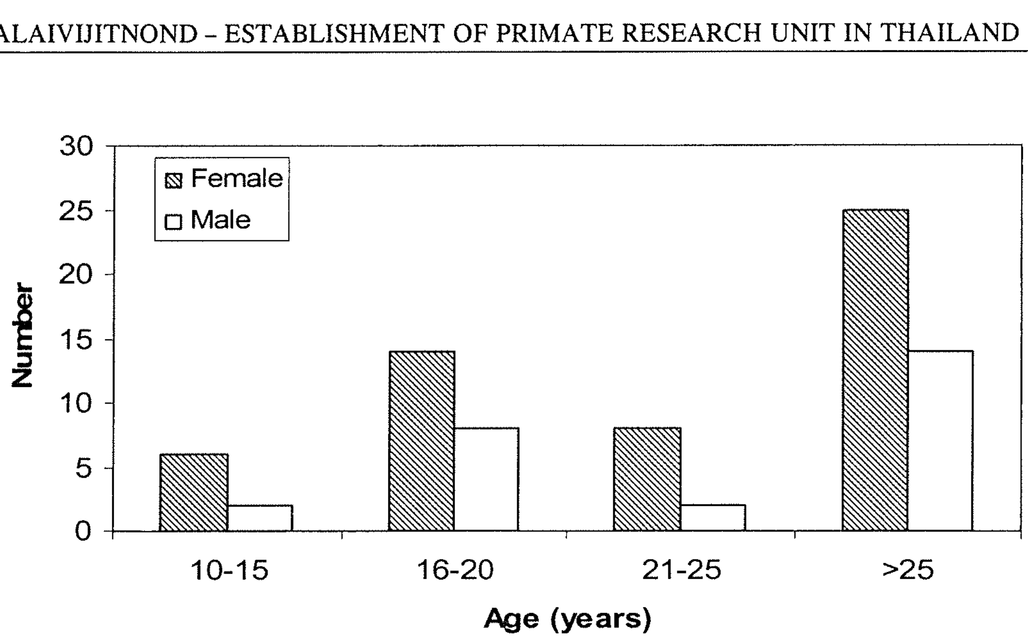

FIGURE 2. Number of various ages of long-tailed macaques at the Primate Research Unit. Horizontal line indicates the age of menopausal or andropausal stage.

the research on biodiversity of macaques will be expanded from domestic to Southeast Asian countries, e.g. Laos, Vietnam, Myanmar and Cambodia. Thus, it is prerequisite to construct the international network among Southeast Asian Primatologists, and the Primate Research Unit will be an important center for such communication.

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The Current Distribution and Status of Macaques in Thailand

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ABSTRACT.- **The current distribution and status of five macaque species, *Macaca fascicularis, M. mulatta, M. arctoides, M. assamensis and M. nemestrina*, in Thailand were determined by questionnaire survey in the sub-district level and by field surveys. Among these 5 species of macaques, long-tailed macaques are the most successful species adapted to a wide range of ecological habitats in Thailand. We found this species at 68 localities during our survey, but in contrast at only 5- 10 localities for each of the other four species. In those troops where it was practically feasible, monkeys were temporarily caught, and data and samples were collected for morphological, physiological, and genetic analyses. Based on our analyses, long-tailed macaques have a wide geographical range in Thailand (from *ca.* 6° N to 16° N), and they can be lumped into either Indochinese or Sundaic groups separated by the boundary at the Isthmus of Kra. Two troops of *M. fascicularis aurea* were found on islands just below the Isthmus of Kra. The probable hybrid monkeys between long-tailed and rhesus macaques were found at the postulated hybrid zone (15-20° N). The severe threats to Thai macaque populations are habitat deterioration and the artificial genetic pollution caused by pet release and monkey translocation.**

KEY WORDS: Status, Distribution, Macaque, Thailand

INTRODUCTION

Macaque monkeys (the genus *Macaca*) are one of Cercopithecoid taxa and are mainly distributed in Asia. Thailand is located at the center of the Indochinese peninsula and occupies an important part in biogeography and biodiversity, functioning as the connecting corridor for fauna and flora migration between continental Asia and the archipelago parts of Southeast Asia (so-called "Sunda land", that is formed in the Sunda shelf, including Sumatra, Java, Bali, Kalimantan, Palawan, and some smaller islands in the vicinity). Thus, Thailand is an important area for the study of the evolutionary history of macaque monkeys.

There are five species of macaque monkeys in Thailand; *M. arctoides* (stump-tailed macaque), *M. assamensis* (assamese macaque), *M. nemestrina* (pig-tailed macaque), *M. mulatta*

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(rhesus macaque) and *M. fascicularis* (long-tailed macaque), representing an example of each of the four species-groups recognized by Fooden's classification (Fooden, 1976); *M. arctoides* to *arctoides* species-group, *M. assamensis* to *sinica* species-group, *M. nemestrina* to *silenus-sylvanus* species-group, and *M. mulatta* and *M. fascicularis* to *fascicularis* species-group. Currently, there is no comprehensive data on the physiology and ecology of macaque monkeys in Thailand, and very little data on their morphology (Suzuki and Varavudhi, 1989) and population genetics (Kawamoto et al., 1989; Harihara et al., 1991). Most of the literature concerns their distribution and is derived from the surveys carried out more than 15 years ago (Fooden, 1971; Aggimarangsee, 1992), and so is in desperate need of updating given the significant level of recent human activity in this region

Nowadays, humans have invaded and disturbed the natural habitat of macaques by forest destruction, rapid industrial development, new road construction and wide spread encroachment. The monkey troops have been isolated from each other with consequently of the risk of increased inbreeding depression. Additionally, the fragmentation and isolation of natural vegetation and creation of small habitat areas have forced different macaque species to share the same constrained habitat and so increase the production of cross-species hybrids, which are fertile in macaques (Bernstein and Gordon, 1980). In addition to such natural hybridization, another serious threat to macaques in Thailand is the human-made hybridization. Thai people transporting and rearing macaques as pets usually release them into the vicinity of wild- or semi-wild macaque troops resulting in the production of novel genetic hybrid offspring (Malaivijtinond et al., 2004). Therefore, to understand the importance of and to conserve the genetic integrity of macaque species and isolates (such as biotypes) in Thailand, it is urgently needed not only to prevent such 'artificial" hybrids (genetic pollution), but also to obtain the basic knowledge and up-dated information on distribution, population (size and structure), morphology, physiology, ecology and population genetics. To this aim we are, therefore conducting research on the five Thai macaque species to collect data on the distribution and present status of local troops and their habitat conditions throughout Thailand by questionnaire based and direct filed observation based surveys. In troops where it is practically feasible, a capture-and-release based field study was conducted to collect samples and data on the morphology, physiology, parasitology and population genetics. In this manuscript we report the preliminary results of this ongoing research.

MATERIALS AND METHODS

1. Questionnaire Survey

The questionnaire asking for 13 species of primates in Thailand (Lekagul and McNeely, 1988), including macaque monkeys, were sent with an explanatory brochure and stamped reply envelope to the head of each Tambon (Sub-district), so-called Kamnan, throughout Thailand (7,394 Tambons). The address of each Tambon was accessed from the website "http://www.Thaitambon.com"

2. Field Survey

Those Tambons from which questionnaires were replied were selected to directly examine the distribution, population (size and structure), physiology and morphology of all five species of macaques. Both morphological (e.g. patterns of crest, crown, direction of hair at check (ridge of hairs or whorl), and shape of ischial callosity, shape of ear, hair colour, body proportions, body size and tail length) and physiological characters (e.g. swelling and reddening of sexual skin in female monkeys) were observed and recorded.

3. Capture-and-release field work

After field survey, monkeys from localities selected as practically feasible for trapping were temporarily caught in a trap (6 m x 6 m x 1.5 m or 6 m x 10 m x 1.5 m). Monkeys were anesthetized by intramuscular injection with 10 mg/kg BW of ketamine hydrochloride. While the monkeys were immobilized, somatometrics (Iwamoto, 1971) and pelage color (Hamada et al., 1988; 1992) were recorded, palmar and plantar dermatoglyphics (Iwamoto and Suryobroto, 1990) and photos were taken, fecal (Saksirisampant et al., 2003) and blood samples (Malaivijitnond et al., 2002) were collected, and overall health was examined. The monkeys were released back to the troop after their complete recovery. The ages of monkeys were estimated from the dental formula according to Smith et al. (1994). So far three groups of *M. mulatta* in the northeastern region and ten groups of *M. fascicularis* throughout Thailand have been inspected.

4. DNA amplification and sequence determination

DNA was extracted from the blood buffy-coat following the method described by Sambrook et al. (1989). The sequences of a *ca.* 450 bp non-coding region of mtDNA (Hayasaka et al., 1991) and the TSPY gene (Kim and Takenaka, 1996; Tosi et al., 2000) were analyzed. The DNA samples of macaques originated from SE Asian countries collected by O. Takenaka were also included in the phylogenetic analysis.

REsults AND DISCUSSION

1. Questionnaire survey

Replies to the questionnaire were received from Tamboons, representing a *ca.* 15% return. Some of replies gave additional and valuable information that had not been anticipated.

2. Field survey

The field surveys currently cover 500 sites, which represents 40 percent of the localities from the replied questionnaire. The data to hand suggests that the misidentification of macaque species varied with regions, as expected. In the northern and central regions, misidentification between long-tailed and rhesus macaques were evident. In the northern and western regions, probable misidentification between rhesus and assamese macaques was found. However, the identification of macaque species by the southern people was believed to be more reliable and mostly correct. Possibly, the sympatric distributions between long-tailed and rhesus macaques in the northern and central regions, and between rhesus and assamese macaques in the northern and western regions (Fooden, 1982a) have caused these misidentifications. This scenario is likely because the relative tail-length, pelage color and body mass between long-tailed, rhesus and assamese macaques in Thailand are not always definitively different between each other at a level clearly distinguishable that local Thai people (non-macaque taxonomists) can perceive. Hamada et al. (2005a; 2005b) reported that the relative tail-length of rhesus macaques from northeastern Thailand was much longer (54-70 %) than those from China (36%) and India (42%), whereas the body mass was much lower. On the other hand, three sympatric species of macaques, long-tailed, stump-tailed and pig-tailed macaques, in the south showed extensively different morphological characters, and the local people in the south are very familiar with pig-tailed macaques since they keep them for coconut-picking. The names and geographical co-ordinates obtained by Global Positioning System (GPS) of the localities where we found the macaques are listed in Table 1.

3. Preliminary results on the distribution and present status of macaques in Thailand

*Long-tailed macaques (*Macaca fascicularis*)*

Among the five species of macaques in Thailand, long-tailed macaques are the most frequently observed, perhaps a reflection of their wide adaptability to various ecological conditions including human habitations. They are found in primary forests, disturbed and secondary forests, riverine and coastal forests of nipa palms and mangroves, urban villages, public parks, and temples. We found this species at 68 localities (approximately 14 % of all localities surveyed) during our survey, 31 of which (46%) are situated in the southern region. Many troops in the south were found at just two localities, Ranong (7 troops) and Nakhonsrithammarat Provinces (6 troops). The distribution pattern of long-tailed macaques at present appears broadly similar to that surveyed 30 years ago (Fooden, 1995), being distributed from the lower northeast and north (Mahasarakham and Nakhonsawan Provinces; c.a. 16° N) to the southernmost parts (Yala Province; c.a. 6o N). However, their habitats have significantly changed in this time from natural forests to temples or parks (Malaivijitnond et al., 2004), and thus their habitat shows a new human association. Additionally, with the intense provisioning, the population has increased by about 25 - 775% compared to the estimated levels of 15 years ago (Aggimarangsee, 1992).For example, troop Nos. 8, 9, 13, 15 and 21 were comprised of more than 1,000 individuals.

Here, we describe some special features found in some troops.

Troop No.1 (Kumpawaphi City Park) showed mixed characters of *M. fascicularis, M. mulatta* and *M. nemestrina.* The habitat lies along latitude 15-20° N, a postulated hybrid zone between long-tailed and rhesus macaques (Fooden, 1995). It is thus hypothesized that male rhesus macaques immigrated into the troops of long-tailed macaques (Malaivijitnond et al., 2005a). The characteristics of pig-tailed macaques in troop No. 1 were possibly introduced by hybridization with released pet pig-tailed macaques (data derived from interviews with local people). We also found a troop of monkeys at the Khao Khiao Open Zoo (No. 33) with mixed characters of long-tailed and rhesus macaques. This is located on the mountain at 13° N, which is far from "the postulated hybrid zone". By interviewing the Veterinarian at the Zoo, it was ascertained that there have been troop(s) of native long-tailed macaques on that mountain before the Zoo was established in 1974, and it is supposed that pet or captive rhesus macaques were released into the native long-tailed macaque troop. We also found also a released pig-tailed macaque in troop No. 9 (Wat Tasang Tai), and released female stump-tailed and female pig-tailed macaques in troop No. 15 (San Prakan).

Troop No. 2 (Wat Pa Sila Wiwek, Mukdahan Province), the northernmost *M. fascicularis* troop (16° 32' 63.1" N), was translocated from troop No. 4 (Don Chao Poo Forest Park, Amnat Chareon Province; 15° 36' 36.4" N), approximately 100 km away, by the monks.

Two pubertal females in the troop No. 4 exhibited a scrotum-like feature at the inguinal part. We also found female monkeys with a similar feature in troop No.21 (Khao Ngu Rock Garden). However, eight out of the 33 female monkeys captured showing a scrotum-like feature were confirmed as females by analysis of amelogenin and SRY genes.

Troop No. 3 (Kosumphi Forest Park) is located in the northernmost region (16° 15' 13.0" N). Some individuals in the troop showed a golden pelage ("Golden" macaques, Hamada et al., 2005c).

From interviews with the local people and chief monk at Wat Tasang Tai, Nakhonsawan Province, troop No. 8 (Wat Khao Nor; 15° 57' 01.5" N) was found to have originated from troop No. 9 (Wat Tasang Tai; 15° 56' 53.8" N). some members of troop No. 9 migrated across the Ping River (tributary of Chaophraya River) and established the troop No. 8 for 15 years ago.

Based on the analyses on physiological, morphological and genetic characters, long-tailed macaques in Thailand can be lumped into either Indochinese (north) or Sundaic (south) groups. The boundary between the two groups is at the Isthmus of Kra, Ranong province (10° 28' 17.3" N, 98° 48' 36.8" E), a major zoogeographical boundary. The Indochinese group has a longer crown-lump length and a shorter tail compared to the Sundaic group. As for the physiological characters, monkeys inhabiting the Sundaic region (from troop No. 29 to the troop No. 68 in our survey) showed the swelling and reddening of sexual skin at the base of the tail, but this was absent in the Indochinese monkeys. The result of genetic analyses of 450-bp of mtDNA sequences revealed that Indochinese monkeys are monophyletically separated from the Sundaic monkeys. The latter monkeys were further separated into 2 subgroups, Mainland (Thailand) and Island (Philippines, Sumatra, and Java), except for monkeys of troop No. 68 (Wat Khuha Phimuk, Yala Province, which did not cluster with Sundaic monkeys but with the group of Malaysian monkeys.

It has been suggested that sub-specific hybrids between *M. f. fascicularis* and *M. f. aurea* (mainly distributed in Myanmar) occurred in the restricted area north to the Isthmus of Kra (Fooden, 1995). During our survey, we found macaques showing characters of M. f. aurea in troop Nos. 40 and 44 from Ranong province.

TABLE 1. Names and geographical co-ordinates obtained using the Global Positioning System (GPS) for the five species of macaques found in Thailand. Wat, Ban, Khao, and Koh stand for temple, village, mountain and island, respectively, in Thai.

Macaca fascicularis

Region	Name of Location	GPS
Northeast	1[a]. Kumpawaphi City Park, Amphoe Kumpawaphi, Udon Thani Province	N 17° 06' 37.5", E 103° 01' 14.7"
	2[b]. Wat Pa Sila Wiwek, Amphoe Muang, Mukdahan Province	N 16° 32' 63.1", E 104° 43' 43.2"
	3[c]. Kosumphi Forest Park, Ban Khumklang, Tambon Huakwang, Amphoe Kosumphisai, Mahasarakham Province	N 16° 15' 13.0", E 103° 04' 18"
	4[d]. Don Chao Poo Forest Park, Tambon Phana, Amphoe Phana, Amnat Chareon Province	N 15° 36' 36.4", E 104° 50' 53.1"
	5. Wat Kupra Kona, Tambon Srakhu, Amphoe Suwannaphum, Roi-et Province	N 15° 33' 16.7", E 103° 49' 26.9"
	6. Muangling Ban Whan, Tambon Ban Whan, Amphoe Suwannaphum, Srisaket Province	N 15° 22' 44.4", E 104° 10' 59.4"
	7. Wat Ban Muangkhaen Potharam, Tambon Muangkhaen, Amphoe Rasi Salai, Srisaket Province	N 15° 21' 35.2", E 104° 13' 10.2"
North	8. Wat Khao Nor, Tambon Ban Daen, Amphoe Banphotpisai, Nakhonsawan Province	N 15° 57' 01.5", E 99° 52' 56.6
	9. Wat Tasang Tai, Tambon Tasang, Amphoe Banphotpisai, Nakhonsawan Province	N 15° 56' 53.8", E 99° 57' 15.6"
	10.Wat Paa Khao Pha, Ban Wangtien, Tambon Khokprong, Amphoe Wichianburi, Petchabun Province	N 15° 47' 51.7", E 101° 13' 34.8"
	11. Wat Tham Thepbandan, Tambon Samyaek Wichianburi, Amphoe Wichianburi, Petchabun Province	N 15° 44' 51.0", E 101° 02' 20.0"
	12. Wat Kriang Krai Klang, Tambon Kriang Krai, Amphoe Muang, Nakhonsawan Province	N 15° 44' 02.3", E 100° 11' 33.6"
Central	13. Wat Khao Patthawee, Tambon Taluk-khoo, Amphoe Tubtan, Uthai Thani Province	N 15° 28' 22.1", E 99° 45' 31.4"
	14. Wat Phikun-ngam, Tambon Khungsampao, Amphoe Manorom, Chainat Province	N 15° 16' 16.6", E 100° 03' 36.3"
	15. San Prakan, Amphoe Muang, Lobburi Province	*c.a.* N 14° 47', E 100° 27.0'
	16. Wat Kai, Ban Wat Kai, Tambon Hansang, Amphoe Bangpahan, Ayutthaya Province	N 14° 30' 35.5", E 100° 31' 22.3"
	17. Wat Hansang, Amphoe Bangpahan, Ayutthaya Province	*c.a.* N 14° 30', E 100° 31'
	18. Wat Praputthachai, Amphoe Muang, Saraburi Province	*c.a.* N 14° 28', E 100° 56.3'
	19. Forest close to Mahidol University of Kanchanaburi Campus, Amphoe Saiyok, Kanchanaburi Province	N 14° 07' 24.0", E 99° 09' 18.0"
	20. Wat Thammasala, Tambon Thammasala, Amphoe Muang, Nakhonpathom Province	N 13° 48' 42", E 100° 06' 52"
	21[d] Khao Ngu Rock Garden, Tambon Khao-ngu, Amphoe Muang, Ratchaburi Province	N 13° 34' 19", E 99° 46' 20"
	22. Bangtaboon Witthaya School, Ban Bangtaboon-oak, Amphoe Banlaem, Petchaburi Province	*c.a.* N 13° 15', E 99° 57'
	23. Wat Tham Khao Yoi, Ban Khao Yoi, Tambon Khao Yoi, Amphoe Khao Yoi, Petchaburi Province	N 13° 14' 05.8", E 99° 49' 31.0"
	24. Pranakhonkhiri or Khao Wang, Amphoe Muang, Petchaburi Province	*c.a.* N 13° 08', E 99° 58'
	25. Wat Khao Luang, Tambon Thongchai, Amphoe Muang, Petchaburi Province	N 13° 07' 59.5', E 99° 55' 50.0"
	26. Wat Khao Thamon, Ban Khaothamon, Tambon Thasen, Amphoe Banlad, Petchaburi Province	N 13° 02' 50", E 99° 57' 04"
	27. Wat Khao Krachiw or WatBanpatawad, Tambon Thayang, Petchaburi Province	N 12° 57' 40.4", E 99° 54' 47.2"
	28. Sam Roi Yot National Park, Tambon Khao Daeng, Amphoe Kuiburi, Prachuab Khirikhan Province	N 12° 07' 30.6", E 99° 57' 09.1"
	29. Wat Khao Takieb, Tambon Nongkae, Amphoe Hua Hin, Prachuab Khirikhan Province	N 12° 30' 51.4", E 99° 59' 09.4"
	30. Wat Thammikaram Worawiharn or Wat Khao Chong Krachok, Amphoe Muang, Prachuab Khirikhan Province	N 11° 48' 41.4", E 99° 48' 05.3"

TABLE 1. (continued)

Macaca fascicularis

Region	Name of Location	GPS
East	31. Wildlife Research Center, Khao Ang Luea Nai Wildlife Reserve, Chachoengsao Province	c.a. N 13° 24', E 101° 52'
	32. Khao Sam Muk, Tambon Saensuk, Amphoe Muang, Chonburi Province	N 13° 18' 45.0", E 100° 54' 14.5"
	33e. Khao Khiao Open Zoo, Tambon Bangpra, Amphoe Sriracha, Chonburi Province	N 13° 12' 56.0", E 101° 03' 19.7"
	34. Wat Khao Cha-ang-on Songkhuang, Tambon Puangthong, Amphoe Bo Thong, Chonburi Province	N 13° 12' 29.6", E 101° 39' 05.5"
	35. Wat Khao Cha-ang-on Nok, Tambon Puangthong, Amphoe Bo Thong, Chonburi Province	N 13° 06' 52.4", E 101° 34' 54.2"
	36. Small hill close to Sudthangruk Restaurant, Tambon Thung Sukhla, Amphoe Sriracha, Chonburi Province	N 13° 04' 35.4", E 100° 52' 30.4"
	37. Lan Hinkhong Monastery or Khao Phlutaluang Monastery, Tambon Phlutaluang, Amphoe Sattahip, Chonburi Province	N 12° 42' 15.5", E 100° 58' 00.3"
South	38. Nong Yai Water Reservoir, Tambon Banglauk, Amphoe Muang, Chumphon Province	N 10° 32' 59.9", E 99° 12' 26.8"
	39. Wat Tha Mailai, Tambon Wangmai, Amphoe Muang, Chumphon Province	N 10° 29' 43.3", E 98° 57' 27.4"
	40f. Wat Paknam Pracharangsarith, Tambon Paknam, Amphoe Muang, Ranong Province	N 9° 57' 11.8', E 98° 35' 41.4"
	41. Suan Somdet Prasrinakharin Chumphon, Tambon Thamapla, Amphoe Lhangsuan, Chumphon Province	N 9° 56' 52.4", E 99° 02' 21.5"
	42. Ban Ko Lhao, Tambon Paknam, Amphoe Muang, Ranong Province	N 9° 54' 28.1", E 98° 34' 05.3"
	43. Mangrove Research Center, Tambon Ngao, Amphoe Muang, Ranong Province	N 9° 52' 38", E 98° 36' 09.6"
	44f. Koh Piak Nam Yai and Koh Thao, Laemson National Park, Ban Bangben, Tambon Muangkuang, Amphoe Kapur, Ranong Province	N 9° 35' 01.1", E 98° 28' 22.9"
	45. Water Reservoir, Moo 5, Tambon Ban-na, Amphoe Kapur, Ranong Province	N 9° 32' 48.8", E 98° 42' 27.8"
	46. Wat Tham Silatieb, Moo 1, Tambon Wang, Amphoe Thachana, Surat Thani Province	N 9° 31' 00.5", E 99° 11' 28.4"
	47. Eastern Marine Research Center, Ban Kamphuan, King-Amphoe Suksamran, Ranong Province	N 9° 22' 20.2", E 98° 24' 9.4"
	48. Pak Nam Laempho, Ban Laempho, Tambon Phumriang, Amphoe Chaiya, Surat Thani Province	N 9° 22' 30.3", E 99° 15' 56.9"
	49. Ban Pak Nam 1, Moo 3, Tambon Thakham, Amphoe Phunphin, Surat Thani Province	N 9° 05' 46.3", E 99° 13' 56.5"
	50. Decha Tukhan Monastery, Ban Khao Tasak, Amphoe Sichon, Nakhonsrithammarat Province	N 9° 04' 31.8", E 99° 50' 21.2"
	51. Koh Klang, Tambon Bang Muang, Amphoe Takua Pa, Ranong Province	N 8° 52' 12.1", E 98° 21' 00.2"
	52. Wat Nasarn, Ban Nasarn, Tambon Nasarn, Amphoe Nasarn, Surat Thani Province	N 8° 48' 30.4", E 99° 22' 10.6"
	53. Wat Ban Rhiang, Tambon Ban Rhiang, Amphoe Thuppud, Phang-nga Province	N 8° 35' 49.7", E 98° 40' 35.2"
	54. Wat Tham Tapan, Tambon Taychang, Amphoe Muang, Phang-nga Province	N 8° 27' 16.3", E 98° 31' 40.2"
	55. Wat Suwankhuha, Amphoe Takhua Thung, Phang-nga Province	N 8° 25' 38.9", E 98° 28' 27.6"
	56. Srinakharinthara Princess Garden, Amphoe Muang, Phang-nga Province	N 8° 25' 59.8", E 98° 31' 13.2"
	57. Ban Chong Mai Kaew, Tambon Khao Yai, Amphoe Au Luk, Krabi Province	N 8° 24' 48.3", E 98° 44'11.8"
	58. Moo 4, Ban Kong Khong, East Pak Phanang, Amphoe Pak Phanang, Nakhonsrithammarat Province	N 8° 23' 59.1", E 100° 11' 59.7"
	59. Wat Chay Khao, Tambon Khao Kaew, Amphoe Lansaka, Nakhonsrithammarat Province	N 8° 21' 19.7", E 99° 47' 41.8"
	60. Wat Khuhasantayaram or Wat Tham Khao Daeng, Moo 9, Tambon Hintok, Amphoe Ronphibun, Nakhonsrithammarat Province	N 8° 14' 29.1", E 99° 52' 11.6"
	61. Wat Khao Kaew Wichian, Tambon Chianyai, Amphoe Chianyai, Nakhonsrithammarat Province	N 8° 12' 02.1", E 100° 05' 52.4"
	62. Ban Kho-en, Tambon Mai Khaw, Amphoe Thalang, Phuket Province	N 8° 09' 43.0", E 98° 20' 21.2"
	63. Khao Chumthong Monastery, Moo 2, Tambon Thungpho, Amphoe Chulabhorn, Nakhonsrithammarat Province	N 8° 08' 1.0", E 99° 52' 36.4"
	64. Wat Thamsue, Tambon Krabinoi, AMphoe Muang, Krabi Province	N 8° 07' 18.6", E 98° 55' 39.8"
	65. Wat Khuha Sawan, Tambon Khuha Sawan, Amphoe Muang, Phatthalung Province	N 7° 37' 4.1", E 100° 5' 2.0"
	66. Khao Chaison, Amphoe Khao Chaison, Phatthalung Province	c.a. N 7° 30', E 100° 10'
	67. Khao Noi/Khao Tangkuan, Amphoe Muang, Songkhla Province	N 7° 12' 33.2", E 100° 35' 49.8"
	68. Wat Khuha Phimuk, Tambon Natham, Amphoe Muang, Yala Province	c.a. N 6° 31', E 101° 13'

a = Monkeys showed mixed characters of *M. fascicularis*, *M. mulatta* and *M. nemestrina*.
b = Monkeys in this troop were translocated from troop No. 4, Don Chao Poo Forest Park, Amnat Chareon Province
c = Monkeys with golden pelage color were found in this troop.
d = Scrotum-like female monkeys were found in these troops.
e = Monkeys showed mixed characters of *M. fascicularis* and *M. mulatta*.
f = Monkeys showed the morphological sub-specific characters of *M. fascicularis aurea*.

TABLE 1. (continued)

Macaca mulatta

Region	Name of Location	GPS
Northeast	1. Wat Pa Aranwiwek-Phusing, Ban Nayao, Tambon Chaiyaporn, Amphoe Buangkan, Nongkhai Province	N 18° 12’ 27.1", E 103° 50’ 00.5"
	2. Wat Pa Banpaum, Ban Paum, Tambon Nakhae, Amphoe Nayung, Udon Thani Province	N 18° 00’ 47.3", E 102° 05’ 10.5"
	3. Wat Pa Phukon, Ban Nakham, Tambon Bankong, Amphoe Nayung, Udon Thani Province	N 17° 54’ 54.9", E 102° 07’ 20.2"
	4. Wat Udommongkolwanaram (Wat Pha Nakham Noi), Tambon Nakong, Amphoe Nayung, Udon Thani Province	N 17° 54’ 14.4", E 102° 10’ 03.8"
	5. Ban Sang School, Ban Sang, Tambon Sang, Amphoe Seka, Nongkhai Province	N 17° 51’ 27.4", E 103° 57’ 46.7"
	6. Wild Monkey Park, Ban Phon Koh, Tambon Nakham, Amphoe Srisongkhram, Nakhon Phanom Province	N 17° 39’ 29.8", E 104° 19’ 58.3"
	7. Wat Phattanajit, Ban Nongdu, Tambon Nanai, Amphoe Phonsawan, Nakhon Phanom Province	N 17° 26’ 08.5", E 104° 34’ 23.1"
	8[a]. Wat Tham Pa Mak Ho, Amphoe Wangsapung, Loei Province	N 17° 14’ 05.6", E 101° 46’ 80.8"
	9. Wat Tham Sung-Tham Pha Nang Khoi, Ban Jomthong, Tambon Phuanphu, Amphoe Nong Hin, Loei province	N 17° 04’ 51.5", E 101° 47’ 14.7"
	10. Phu Khiao National Park, Amphoe Khonsarn, Chaiyaphum Province	N 16° 27’ 38.3", E 101° 39’ 22.2"

a = A hybrid juvenile was sired by a released *M. nemestrina* male.

Macaca arctoides

Region	Name of Location	GPS
Central	1[a]. Thungyai National Park, Uthaithani Province	*c.a.* N 15° 26’, E 98° 44’
	2[a]. Huai Kha Khaeng Wildlife Sanctuary, Uthaithani Province	*c.a.* N 15° 17’, E 99° 10’
	3[b] Wat Khao Krapuk, Tambon Khao Krapuk, Amphoe Thayang, Petchaburi Province	*c.a.* N 12° 51’, E 99° 41’
	4[a]. Khao Krapuk-Khao Taomo Non-hunting area, Tambon Kadluang, Amphoe Thayang, Petchaburi Province	N 12° 47’ 46.0", E 99° 44’ 42.4"
South	5. Wat Tham Khaowong, Ban Hup Khaowong, Tambon Kamnerdnoppakhun, Amphoe Bangsaphan, Prachuab Khirikhan Province	N 11° 17’ 28.0", E 99° 29’ 46.2"
	6[a]. Khao Sok National Park, Suratthani Province	*c.a.* N 9° 00’, E 98° 30’
	7. Wat Tham Tapan, Tambon Taychang, Amphoe Muang, Pang-nga Province	N 8° 27’ 16.3", E 98° 31’ 40.2"
	8[c]. Khao Luang National Park, Nakhonsithammarat Province	*c.a.* N 8° 24’, E 99° 48’
	9[d]. Wat Tham Khao Daeng or Wat Khuhasantayaram, Tambon Hintok, Amphoe Ronbibun, Nakhonsithammarat Province	N 8° 14’ 37.4", E 99° 52’ 00.9"

a, b and c = *M. arctoides*’ s troops reported by Treesucon (1988), Aggimarangsee (1992) and Boonratana (1988), respectively.

d = The locality where *M. arctoides* are sympatric with *M. fascicularis* (Malaivijitond and Hamada, 2005b)

Macaca assamensis

Region	Name of Location	GPS
North	1. Wat Tham Pla, Amphoe Maesai, Chinagrai Province	N 20° 19’ 44.4", E 99° 51’ 45.3"
	2. Wat Tham Pha Tha Pol Non-hunting area, Amphoe Noen Maprang, Phisanulok Province	N 16° 31’ 01.6", E 100° 40’ 16.0"
	3. Klonglan Waterfall, Klonglan National Park, Amphoe Klonglan, Kamphangphet Province	N 16° 07’ 50.5", E 99° 16’ 37.1"
	4. Um Phang Wildlife Sanctuary, Amphoe Um Phang, Tak Province	*c.a* N 15° 55’, E 98° 49’
West	5. Khao Laem Dam, Tambon Thakhanun, Amphoe Thong Pha Phum, Kanchanaburi Province	N 14° 47’ 49.0", E 98° 35’ 30.6"
	6. Erawan Waterfall, Amphoe Saiyok, Kanchanaburi Province	N 14° 21’ 55.9", E 99° 08’ 48.9"

Macaca nemestrina

Region	Name of Location	GPS
Northeast	1. Wat Tham Kham, Tambon Rai, Amphoe Panna Nikhom, Sakhon Nakhon Province	N 17° 13’ 07.2", E 103° 54’ 02.2"
	2. Khao Yai National Park, Amphoe Pak Chong,Nakhonratchasima Province	c.*a.* N 14° 24’, E 101° 30’
East	3. Khao Chi-on Non-hunting area, Tambon Nachomtaien, Amphoe Banglamung, Chonburi Province	N 12° 46’ 09.5", E 100° 58’ 12.8"
South	4. Wat Tham Khao Marong, Ban Marong, Tambon Pongprasart, Amphoe Bangsaphan Yai, Prachuab Khirikhan Province	N 11° 12’ 08.6", E 99° 29’ 51.9"
	5. Wat Tham Prutakhian, Ban Tapanhin, Tambon Saluy, Amphoe Thasae, Chumphon Province	N 10° 50’ 03.3", E 99° 15’ 38.3"

*Rhesus macaques (*Macaca mulatta*)*

Although we have not surveyed extensively in the north and central regions, we have currently found ten troops of rhesus macaques in the northeastern region (101–104° E). Although many troops are isolated, a large population is continuously deployed along the central longitudinal mountainous area as far as the southern forest limit in the Phu Khiao National Park (troop No. 10). There are different adaptation potentials to the habitat types between long-tailed and rhesus macaques, which is reflected in their distribution pattern. The former appears to adapt to the riverine or disturbed forests better than the latter. On the other hand, the latter seems to prefer mountainous forest, and appears to expand their distribution down to the south along the mountainous areas.

The average relative tail-length of rhesus macaques in Thailand is longer (*ca.* 60%) than the corresponding Chinese (*ca.* 35%) and Indian (*ca.* 45%) populations, but they still conserve the bipartite pattern of pelage color, a unique character to rhesus macaque (Fooden, 1964; Hamada et al., 2005a; 2005b; Fooden, 2000).

Based on the analysis of mtDNA sequences, rhesus macaques in Thailand were separated into 2 groups; rhesus-type (clustered with Indian and Chinese rhesus macaques) and long-tailed-type (clustered with Thai long-tailed macaques). The former group inhabited at 17° 26' to 18° 30' N, and the latter inhabited at 16° 27' to 17° 14' N (Malaivijitnond et al., 2005a).

We found a male pig-tailed macaque was released to the rhesus troop No. 8 (Wat Tham Pa Mak Ho). Being twice as heavy as rhesus adult males, a male pig-tailed macaque became the alpha male and acquired access to reproductively active rhesus females (Malaivijitnond and Varavudhi, 2002). One juvenile male was confirmed to have been sired by a pig-tailed macaque, based on the mtDNA and TSPY gene analysis (Malaivijitnond et al., 2005b).

*Stump-tailed macaques (*Macaca arctoides*)*

Six localities were previously reported for stump-tailed macaques, in the continuous mountain chain or isolated mountains from the central to the southern region (Boonratana, 1988; Treesucon, 1988; Aggimarangsee, 1992). Recently, we found another three troops, troop Nos. 5, 7 and 9, in the south. We found a released female long-tailed macaque in troop No. 4 (Khao Krapuk-Khao Taomo Non-hunting area) during our survey in 2003.

It is of interest that stump-tailed macaques at troop No. 9 (Wat Tham Khao Daeng) are sympatric with a troop of long-tailed macaques by sharing the provisioned food resource (Malaivijitnond and Hamada, 2005). The troop of long-tailed macaques, however, appeared to avoid joint occupancy of the provisioning site with stump-tailed macaques. The pelage and facial skin colour were widely variable between individuals in troop No. 9. The pelage color varied from reddish to light brown to dark brown to blackish in the sub-adult and adult of both sexes. The facial skin color varied from pale pink to dark red to blackish.

*Assamese macaques (*Macaca assamensis*)*

At present, assamese macaques were found at six localities located in the mountainous areas of northern and central regions. Many of their habitats are granite mountains or hills with steep cliffs. As they have tails of middle length (relative tail-length of 30-50%; Fooden, 1982b) and yellowish pelage (but not bipartite pattern as in rhesus macaques), they were occasionally misidentified as rhesus macaques. Principally, differences on size, especially those of limbs, facial morphology, and the pelage color pattern should be stressed for the classification.

*Pig-tailed macaques (*Macaca nemestrina*)*

Although we found many pet pig-tailed macaques, which were reared for coconut picking, during our survey in the south, only in five localities were troops of pig-tailed macaques found. Based on morphological characters, pig-tailed macaques inhabiting the northeastern region of Thailand, represented by troops No.1 and 2 from our survey, are classified as M. nemestrina leonina, and the ones from southern region are classified as the *M. nemestrina nemestrina* (Fooden, 1975). The boundary between the two subspecies is considered around 8-9° N, and probable hybrids were reported in the vicinity of the boundary zone (Fooden, 1975). Although we found mixed characters of the two subspecies in troop No. 5 (Wat Tham Prutakhian, 10° 50' 03.3" N), possibly suggesting hybrids, this was slightly further North than the postulated hybrid boundary. This does not, however, argue against the notion of hybrids.

CONCLUSION

We have recently found more local populations for each of the five macaque species in Thailand than was previously supposed to be existent. However, we also found that their survival and genetic integrity are likely to be under severe threats. First of all, the habitat deterioration by deforestation and agricultural development has made the macaque populations isolated, even though the population sizes are larger than those of the previous reports. Habitat fragmentation and population isolation may reduce gene flow and lead to a decrease in the genetic heterogeneity within populations. In addition to this, the genetic pollution caused by hybridization to translocated and released pet macaques threatens macaques in Thailand. Thai people usually release their pet macaques into the troop of wild or semi-wild macaques when they do not want to rear them anymore. In many localities of Thailand, released pets were found in natural troops of both the same and different species and produced or sired hybrid offspring. Such hybridizations can impair the genetic integrity of the native troop, and makes the evolutionary history of macaques difficult to understand. To conserve the genetic properties of the five macaque species in Thailand, the basic knowledge and up-to-date information on distribution, population (size and structure), morphology, physiology, ecology and population genetics are urgently needed. In addition to such studies the amelioration of habitat and the strict ban of pet release should also be initiated.

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Species Diversity and Distribution of Primates in Myanmar

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ABSTRACT.– One species of prosimians and 13 species of anthropoids are listed as the primate fauna of Myanmar. Diversity of climate, topographies, and the historical exploitation of nature by humans have created the various conditions by which the habitats and population status of Myanmar primates currently exist. Poaching and forest deterioration appear to be serious obstacles to the conservation of primate species. Because systematic and nationwide field research has not yet been conducted; knowledge about the distributions and population status of these primate species is very scarce. To correct this deficit, new records of macaque distributions in the southern part of the Rakhine Mountains were obtained through joint research by the Universities of Yangon and Kyoto in 2004, where five species of macaques, *Macaca assamensis*, *M. arctoides*, *M. mulatta*, *M. fascicularis* and *M. nemestrina* (or *M. leonina*) occur. Further studies on primates promise a greater contribution to the study of the evolution of Asian primates and to the conservation of primates in Myanmar.

KEY WORDS: Primates, Diversity, Distribution, Myanmar

INTRODUCTION

The Union of Myanmar has the largest area (678,600 km^2) of all the countries in Indochina. It stretches for 19 degrees in latitude (Fig. 1), so the landscape of the country is composed of various topographies. The western side of the country is coastal facing the Bay of Bengal and the Andaman Sea. The Rakhine Mountains, which lay along this coast, connect to the eastern Himalayan region in the northern part of Myanmar, where 4000-5000 m peaks of mountains are found (Fig. 2). In the center of the country, vast flood plains extend from the north to the south along the Ayeyarwady and Chindwin Rivers, which run from the Himalayan range. In the eastern part of the country, the Shan plateau delineates the boundary with China, Laos and Thailand. These topographic conditions and the monsoon create a variety of distinctive climates in each region of the country. Variation in climate and topography creates a rich diversity of flora in the country. About 7000 species of plant have been recorded, of which 15% are endemic, and the forest types include mangrove, coastal, swamp, tropical rain and deciduous forests. In the past, forests covered almost all of the

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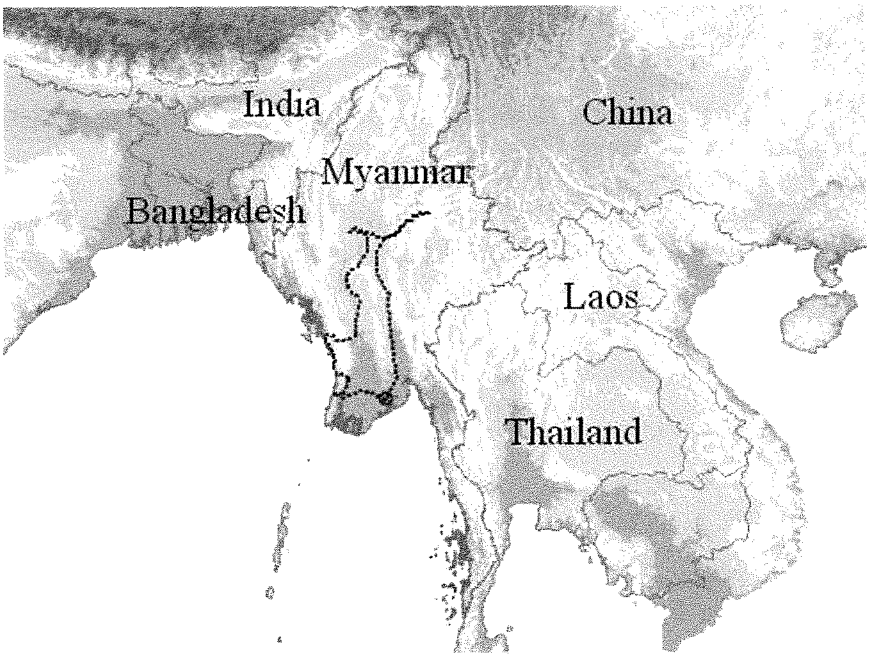

FIGURE 1. The Union of Myanmar and the survey routes in 2004. The dotted line indicates the route.

country but as humans have been exploiting forests heavily, probably since the 11th century, the area of forests has declined to 25000 km^2 at present (Harrison Institute, 2002), and the distribution of mature forests are restricted to only some parts of the Rakhine Mountains, Himalayan region, and Shan plateau.

Myanmar is also rich in fauna, where 1017 species of bird and 245 species of mammal have been recorded (Harrison Institute, 2002). However, the complete fauna of this country has not yet been clarified, especially in areas along the national border. In terms of primates, one species of Prosimian, and 13 species of Anthropoids have been recorded (Yin, 1993). This article aims to report on the present status of primates, trends in primate study and the conservation of primates in Myanmar.

DISTRIBUTION AND PRIMATES

According to Yin (1993), the following 14 primate species inhabit Myanmar: one species of prosimians, Slow loris (*Nycticebus coucang*); five species of macaques, the Assam (*Macaca assamensis*), stump-tailed (*M. arctoides*), rhesus (*M. mulatta*), long-tailed (*M. fascicularis*) and pig-tailed macaques (*M. nemestrina* or *M. leonine*); five species of leaf monkeys, including the banded leaf-monkey (*Presbytis melalophos*), silvered leaf monkey (*Trachypithecus cristatus*), capped leaf monkey (*T. pileatus*), dusky leaf monkey (*T. obscurus*) and Phayre's lutong (*T. phayrei*); and three species of gibbons, the white-handed (*Hylobates lar*) and Hoolock gibbons (*H. hoolock*), and Siamangs (*H. syndactylus*). Yin (1993) gave only general and brief descriptions on the distribution of these primate species in Myanmar (Table 1). Although these species are found in other countries, there are several subspecies endemic to Myanmar. For example, Macaca fascicularis aurea is restricted almost exclusively to Myanmar (Fooden, 1995).

CONSERVATION OF PRIMATES

The Nature and Wildlife Conservation Division, Forest Department and Ministry of Forestry are administratively responsible for the

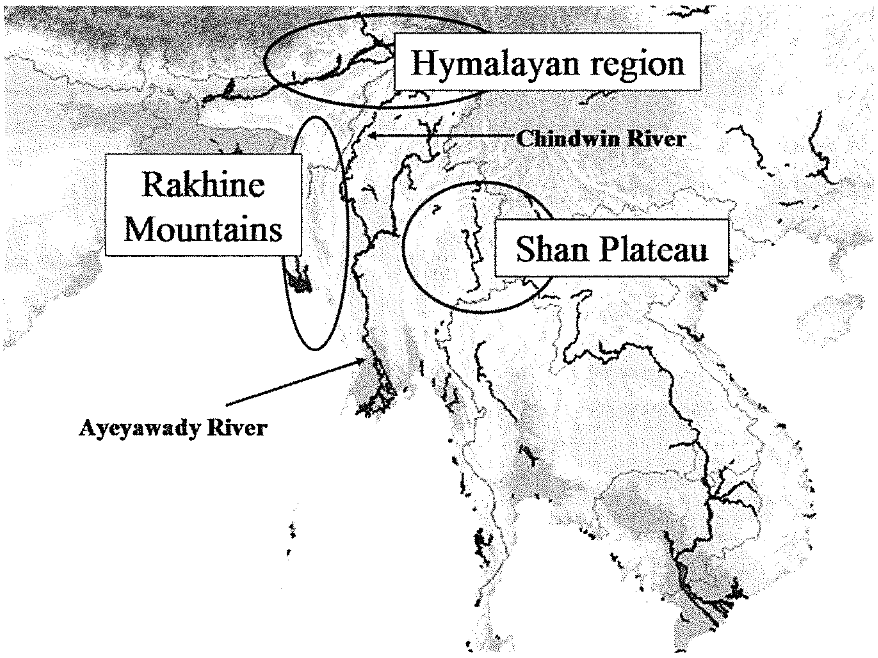

FIGURE 2. The main river systems (bold lines) and mountain areas (within bold ellipsoids, with the range name indicated in bold text within a rectangle) of Myanmar.

conservation of wildlife in Myanmar. All protected areas are owned and managed by the government and are operated under the Protection of Wildlife Law (Law No 6/94 dated June 8, 1994). Through this law 31 nature reservations of 15,270 km^2 (2.3% of the national land) have been designated.

Protected animals (mammals, birds, and reptiles) are listed by the Forest Department. They are classified into three categories according to the necessity of protection: it is strictly prohibited to hunt or breed "completely protected animals", with the exception of those captured for study: for "protected animals", the government permits only controlled kills and live-trapping for breeding purposes; and "seasonally protected animals" can be hunted with government permission during an assigned period but only outside of the protected area. The notification from the Forest Department of the Ministry of Forestry, dated 26 October, 1994, designated the Hoolock gibbon, Siamang, Dusky lutong and Phayre's lutong as "completely protected animals", and other leaf monkeys, macaques, and the slow loris were designated as "protected animals".

Most (85 %) of the people in Myanmar are Buddhist, so the killing or injury of animals in sacred areas, such as a Pagoda, is prohibited. However, outside of a sacred area, hunting by bow gun, gun, and dogs is common, and many of the public are unaware of wild life protection legislation. Hunted animals are consumed directly, used as medicine and exported to neighboring countries, such as China. Furthermore, forest destruction by logging and agriculture is rapidly progressing and the forests are declining by 1.4-2.3% every year (Harrison Institute, 2002). Primate habitats are consequently deteriorating and becoming more fragmented.

STUDY ON PRIMATES

Only a few biologists in Myanmar have shown an interest in the study of primates, and studies by foreign researchers have been rare because of many restrictions to entering local

TABLE 1. Primate fauna in Myanmar and their rough distribution (after Yin, 1993).

Common name	Scientific Name	Range in Myanmar
slow loris	*Nycticebus coucang coucang* *Nycticebus coucang bengalensis* *Nycticebus coucang tenasserimensis*	Myeik Archipelago-King Island Upper Myanmar
pig-tailed macaque	*Macaca nemestrina leonina* or *Macaca leonina*	Upper Myanmar to Tanintharyi, Myeik Archipelago
long-tailed macaque	*Macaca fascicularis aurea*	Lower Myanmar, Tanintharyi, Myeik Archipelago
rhesus macaque	*Macaca mulatta*	Upper Myanmar
Assam macaque	*Macaca assamensis*	Northern Myanmar
stump-tailed macaque	*Macaca arctoides*	Tanintharyi
banded leaf monkey	*Presbyis melalophos*	North Malay Peninsula, Junk Seylon Island, Tanintharyi
silvered leaf monkey	*Trachypithecus cristatus*	Rakhine, Tanintharyi
capped leaf monkey	*Trachypithecus pileatus pileatus* *Trachypithecus pileatus shortridgei* *Trachypithecus pileatus durga*	Garo, Khasi, Jaintia Eastern side of Upper Chindwin South of Naga Hills, Cachar, Tipperah, Chittagong and western side of Upper Chindwin
dusky or spectacled leaf monkey	*Trachypithecus obscurus*	from Northern Malay northwards to Dawei in Tanintharyi
Phayre's leaf monkey	*Trachypithecus phayrei phayrei* *Trachypithecus phayrei shanicus*	Bhamo to Bago North Shan State and their neighbourhood to the east of Ayeyarwady
white-handed gibbon	*Hylobates lar*	Tanitharyi, Rakhine, Lower Bago
Hoolock gibbon	*Hylobates hoolock*	Upper Myanmar, Rakhine
Siamang	*Hylobates syndactylus*	Tanintharyi, Haung Tharaw

areas. However, the Wildlife Conservation Society and Ministry of Forestry have just started an ecological study of Hoolock gibbons with the aim of conservation. Staff at the University of Yangon are carrying out an ecological study on the dusky lutong (or Phayre's lutong) in Mt. Popa Wildlife Sanctuary, Mandalay Division, and have started an ecological study of long-tailed macaques in the Bayin Nyi Naung Mountains, Kayin State. Furthermore, joint research on macaques by the University of Yangon and Primate Research Institute, Kyoto started in 2004.

NEW INFORMATION ON THE DISTRIBUTION OF MACAQUES

Here, we summarize the results of field research on the distribution of macaques conducted in 2004. The genus Macaca is postulated to have originated in Africa 7-8 million years ago (MYA) and deployed its distribution into Eurasia. They reached South Asia at about 5.5 MYA and extended their distribution into Southeast Asia. They finally and rapidly distributed throughout the Far East (Delson, 1980). Speciation occurred several times in some regions, in conjunction with such processes of deployment. Indochina, including Myanmar, is one of the places where such speciation occurred and one of the ecological corridors through which macaques deployed into other areas. In order to elucidate the history of speciation of macaques, we should clarify the geographical distribution of the species at present while analyzing the morphological and genetic variation of species.

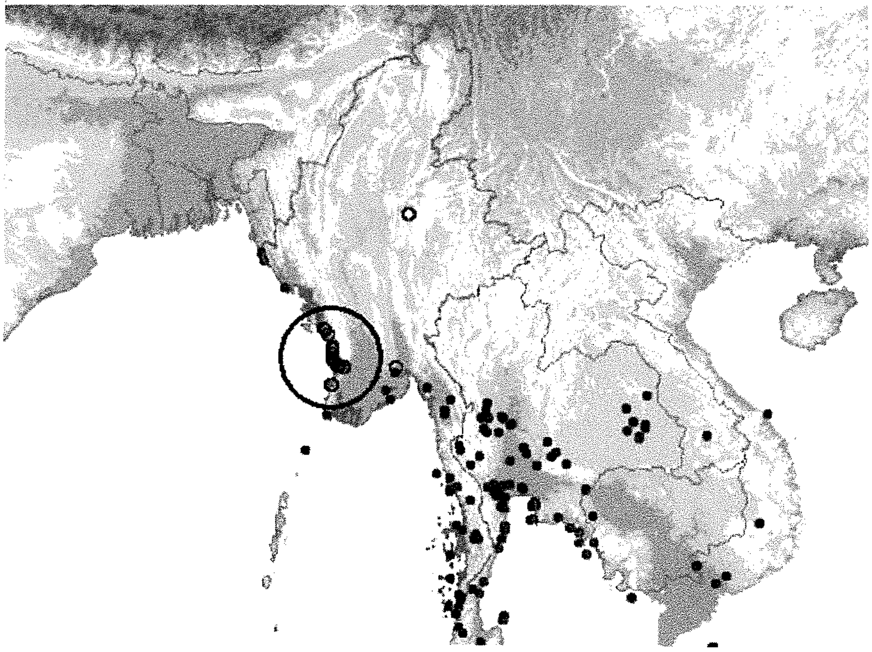

FIGURE 3. New records on the distribution of *Macaca fascicularis* and existing records taken from Fooden (1995). The data from Fooden (1995) is shown as filled circles with new records from the 2004 survey shown in hollow circles. The data enclosed in a bold circle are new records in the Rakhine Mountains.

We conducted a preliminary study on the distribution of macaques in the Union of Myanmar from 12 November to 6 December, 2004. The research areas were some parts of Ayeyarwaddy Division, Rakhine State, Magway Division, Bago Division, Mandalay Division, and Shan State. We interviewed the local people about the presence or absence of each macaque species by showing them photographs that illustrated the typical morphology of the species. We drove 1700 km and conducted interviews at 77 sites along the main road (Fig. 1).

We obtained information on the presence of pig-tailed macaques at 28 sites, long-tailed macaques at 30 sites, rhesus macaques at 49 sites, Assam macaques at 16 sites, and stump-tailed macaques at 6 sites. Of these macaque sites, 19 pig-tailed, 23 long-tailed, 28 rhesus, 11 Assam and 5 stump-tailed were from the southern part of the Rakhine Mountains. This information was obtained from an area where none had previously been available. Thus, they are new records on the presence of macaque species in this area. Nevertheless, because most information was obtained by interviewing the local people (some information was confirmed by observing primates kept as pets), it needs to be reconfirmed by direct field evidence in future studies.

Bearing in mind that the data obtained was principally from interviews of local people and neither macaque biologists nor field studies, and there is thus some uncertainty to its truth, we tentatively show macaque distributions that have not been previously described in reports compiled by Fooden (1975, 1982, 1988, 1990, 1995, 2000). These new findings are that: (1) long-tailed, rhesus, Assam, and stump-tailed macaques occur in the southern part of the Rakhine Mountains and (2) pig-tailed macaques occur in the southern part of the Rakhine and Bago Mountains. Previous reports provided information on long-tailed macaques only in the Malay Peninsular and the eastern part of the Ayeyarwaddy delta. So, our information on

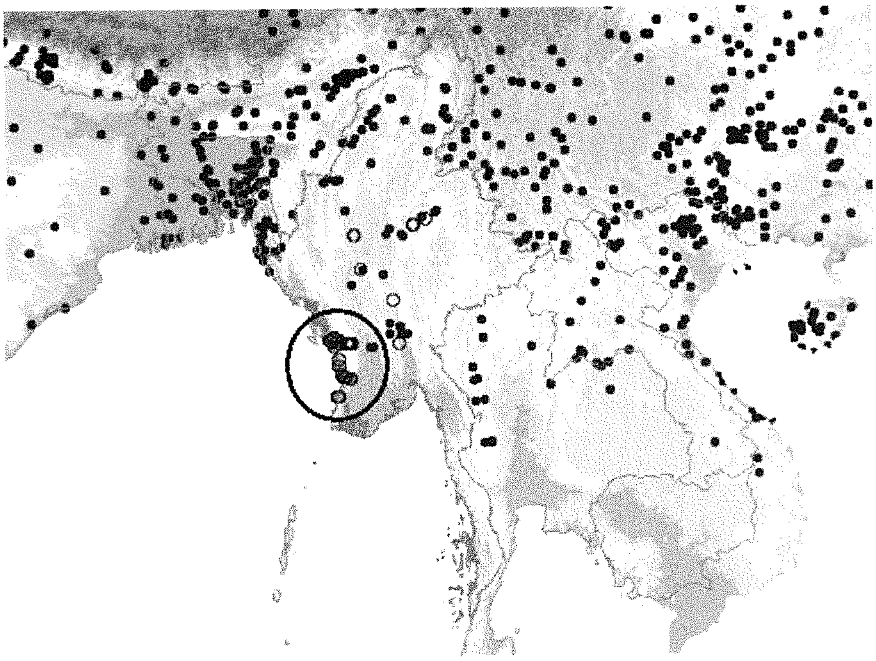

FIGURE 4. New records on the distribution of *Macaca fascicularis* and existing records taken from Fooden (1995). The data from Fooden (1995) is shown as filled circles with new records from the 2004 survey shown in hollow circles. The data enclosed in a bold circle are new records in the Rakhine Mountains.

long-tailed macaques extends their distribution northwards along the coastal forests (Fig. 3). Similarly, previous reports on rhesus macaques provided information only for northern and central Myanmar and the border area of Bangladesh, China, and Thailand (Fig. 4). Assamese and pig-tailed macaques had been reported only in the Shan plateau, and stump-tailed macaques had been known only from the area around the Thai border.

FUTURE STUDY

Myanmar might be one of the last frontiers of zoogeography in Asia because there are many localities where wildlife researchers have never been. The discovery of a new species of barking deer (Muntiacus crinifrons) in 1997 (Rabinowitz et al., 1998) supports this idea. As there is no nationwide research on primate distribution with sufficient precision, clarification of their distribution is needed, especially in the Rakhine Mountains and Shan Plateau where such information is currently unavailable.

Furthermore, setting up a research field to conduct long-term research on ecology, and other comprehensive studies including ecology, morphology and population genetics, is urgently needed, not only to clarify the history of evolution of primates in this country but also to promote the study of conservation of primates and their habitats. The study of primatology in Myanmar is only just starting. We hope to further develop primatology in Myanmar through the cooperation of Universities, such as Yangon and Kyoto, and other organizations.

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Population Explosion of Taiwanese Macaques in Japan

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ABSTRACT.– **A hybrid population between Taiwanese macaques (*Macaca cyclopis*) and Japanese macaques (*Macaca fuscata*) was found in Wakayama prefecture in Central Japan. The distribution and changes in this population were studied. The population was found to increase very rapidly based on three censuses taken over four years. Growth rate of the population was calculated and the future population growth was estimated using the calculated growth rate. The critical points of populations of introduced foreign macaque species and also provisioned macaque populations are discussed.**

KEY WORDS: Taiwanese Macaques, Population growth rate, Hybridization, Introduced species

INTRODUCTION

A population of Taiwanese macaques (*Macaca cyclopis*) was found in Wakayama Prefecture, Central Japan, in 1999 (Kawamoto et al., 2001). This presentation reports on the hybridization between the Taiwanese macaques and Japanese macaques (*Macaca fuscata*) and the explosion of that population.

This Taiwanese macaque population adapted rapidly to the new environment, increased in number and, moreover, hybridized with Japanese macaques (Kawamoto et al., 1999). The hybrid population of Taiwanese macaques is distributed only 20 km apart from the

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original distribution area of Japanese macaques. From the viewpoint of conservation biology two phenomena should be pointed out. The first is the genetic contamination of the population of pure Japanese macaques. As is well known, males of macaques desert their natal troops before maturity, immigrate to neighbouring troops and sire offspring. Thus, male Taiwanese macaques move into troops of Japanese macaques and mate with the females. Given the fertility of cross-species hybrids in macaques the genes of Taiwanese macaques will thus spread into the Japanese macaques. The Hybridization of Japanese macaques with Taiwanese macaques is a crisis for Japanese macaques, which have been endemic to the islands of Japan for three hundred thousand years. This phenomenon is similar to hybridization of red deer with Japanese Sika deer in Britain (Abernethy, 1994).

The second phenomenon to be noted is the disturbance of the ecosystem. Taiwanese macaques may establish a new niche in Japan, although the differences in the niches between Taiwanese and Japanese macaques have not yet been precisely investigated. Taiwanese macaques with their long tail are possibly better adapted to arboreal life or rocky mountains than are Japanese macaques with their short tail. Further, they may be better than Japanese macaques at collecting fruits, insects or bird nests using their long tail as a balancer on the tree, although the function of the longer tail is not yet well analysed (long-tailed macaques vs. pig-tailed macaques (Crockett et al., 1980).

For these critical reasons, the Wakayama prefectural government decided to eliminate all individuals of both Taiwanese and hybrid macaques (Wakayama Prefecture, 2000). The Primate Society of Japan also declared in favour of the plan and offered help to implement it (Primate Society of Japan, 2001).

The purpose of the present study is to elucidate the situation (number, distribution, movement and so on) of the hybrid population and estimate the future population size to help make the eradication of hybrids and Taiwanese macaques successful. The figures of estimated population growth rate were tested in comparison with the demographic data of a provisioned population of Japanese macaques. We discuss population explosion both in provisioned populations and invader populations in rich environments below.

HISTORICAL BACKGROUND OF TAIWANESE MACAQUES IN WAKAYAMA, JAPAN

The population of Taiwanese macaques was supposed to have escaped from a small private zoo at the Ooike area, the peripheral area of Wakayama city around 1950. The number of animals escaped was supposed to be only a few (Conservation Committee PSJ, 2001). Nearly thirty years later on Maekawa (1978) received information about the existence of Taiwanese macaques around the Ooike area. In 1998, one hybrid male Taiwanese / Japanese macaque was found and genetically confirmed as a hybrid by Kawamoto (2001). One month later members (including Ohsawa) of the conservation committee in the Primate Society of Japan detected a hybrid group of more than 20-30 in the Ooike area. In the same year a census of the population was held by the Wakayama prefecture and the (Primatologist) Working Group of Taiwanese macaque, which reported that there were two groups of about 200 animals. The governor decided on elimination of all Taiwanese macaques and their hybrids and started to implement the plan in 2001. The third census was held in 2001 and, although there were still only two groups, the total number had increased to 250 after the capture of 53. The forth census was held in 2004 revealing four distinct groups and a total population of 50-80 after the capture of 295, making an actual population of 345-375.

STUDY AREA AND METHODS

The moving range of the introduced population of Taiwanese macaques locates at the western border of Wakayama city (N. 34°15', W. 135°10'), South of Osaka, Central Japan. The range spreads to around 8.5 square km. The vegetation around the area consists of orchard (peach and mandarin), plantation (bamboo, Japanese cedar) and other miscellaneous vegetation. Mandarin, peach and bamboo shoot are very rich and important food resource for monkeys and therefore they are generally very healthy (based on both anatomical inspection and reproductive data; Unpublished data).

Radio-transmitters were attached to several animals in each group. Radio tracking of troops was intermittently executed and the movement of each group was roughly determined. Systematic survey of the population was held 4 times and we succeeded 3 times in a population census in 1999, 2001 and 2004. The analysis of population data in the present study was based on the 3 successful censuses. The census was conducted by more than 20 members of the working group, by making a census line along a road in the moving ranges of groups where groups cross. We used the Division-method to count the number of each group in the study area. In this method we divided the study area into 19 divisions and allocated several members of the survey in each division. Then we started to detect groups simultaneously in all divisions with the aid of radio-tracking system and also with direct observation, and then summated the number of groups.

RESULTS

The result of the census and the number of captured animals Population sizes and captured and exterminated animal numbers are summarized in Table 1 for the period of the three censuses. The local farmers' organization captured some 50 animals before the second census, thereafter the Wakayama prefecture started systematic capture (Table 1). The calculation of annual population growth rat. We calculated the annual population growth rate based on the census data of 1999 and 2001 only, since the range of the estimated data of 2004 (50-80) was too wide to calculate the growth rate accurately.

TABLE 1. Taiwanese / Japanese macaque hybrid population levels at Wakayama city, Japan

Year	Month	No. captured	Living population size	Total
1999	July		200	200
2001	July	53	250	303
2003	March	15	n/d	
	July	201	n/d	
2004	July	26	n/d	
	September		50-80	50-80

No. captured = the number of hybrids captured and exterminated from the population

Living population size = the estimated population size of living hybrids

Total = the population size (living plus captured and killed)

n/d = not determined

$$N_t = \lambda^t N_0 \text{ then, } \lambda = (N_t / N_0)^{1/t}$$

where N_t: population size in year t,
N_0: population size in year 0
λ: annual population growth rate
t: year.

When we substitute the data to the formula, then $\lambda = (303/200)^{1/(2001-1999)} = 1.14$, which means that the population grows 14% annually. We will examine this figure in comparison with the precisely studied demographic data of a provisioned population of Japanese macaques in the discussion

The estimation of the future population size

The future population size (N_t) can be estimated by substituting the calculated annual growth rate (1.14) to the formula above ($N_t = \lambda^t N_0$). We show the graph until 2006 (Fig. 1). Using this data, we also estimated the population size until 2021 (Fig. 2), assuming no density dependent effects such as disease, competition for food, fighting increased predation etc. That is, as the population gets larger, we still assume a net annual growth rate of 1.14.

DISCUSSIONS

High population growth rate

The population growth of the hybrid population of Taiwanese macaques in Wakayama, Japan revealed itself as seriously high. The Takasakiyama population of Japanese

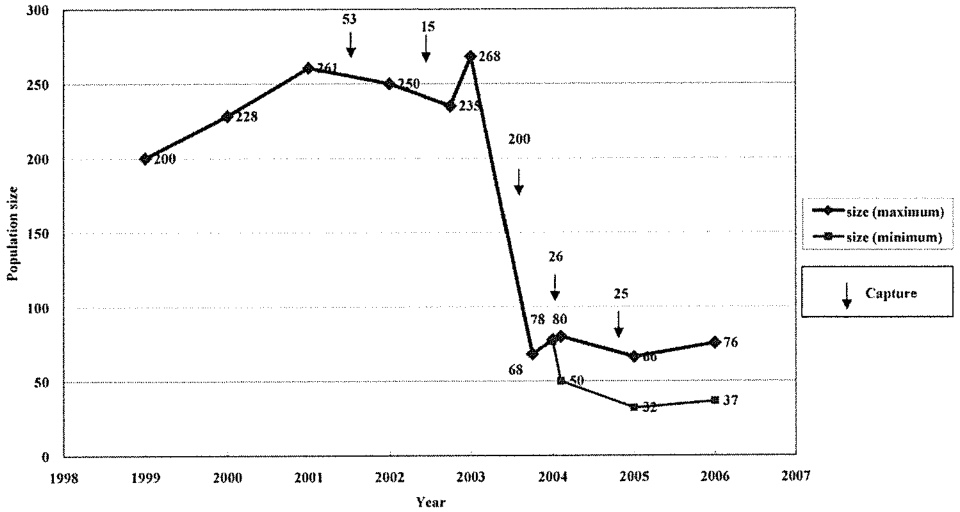

FIGURE 1. Estimation of Population Growth of hybrid Taiwanese / Japanese macaques in Wakayama up to 2006. The minimum and maximum population size predictions are based on the 2004 size population size estimates of 50 – 80, respectively.

macaques (*Macaca fuscata*), which have been well studied, showed an average growth rate of 1.107-1.067 during the period of high level provisioning., whilst the growth rate in a natural population of Yaku Japanese macaques (*Macaca fuscata yakui*) was estimated at 0.92 (Ohsawa 2000 calculated from the data in Takahata et al. (1998), representing a decreasing population size. The highest growth rate physiologically possible in Japanese macaques is estimated as 1.170, where females never die until their maximum longevity (25) and give birth every year after the year of maturity until they die. The figure of 1.14 of the annual population growth rate shown in this hybrid population is near to the physiologically highest growth rata in Japanese macaques (1.17), which occurs only under the conditions of high survival rate, and an almost impossibly high birth rate in a highly provisioned population.

Assuming both no immigration into the populations and that these population size estimates are correct and density independent, we can suppose several causes of this high population growth rate in these hybrid Taiwanese macaques. The first is the rich environment of their habitat. In their moving range there is wide range of orchards of mandarins. The fruiting season of mandarins is in winter and usually many surplus mandarins are discarded in the fields. Macaques can eat them as they want. In general, winter is the severest season for the wild Japanese macaques and sometimes, mass deaths are well known. In the other seasons there are plenty of natural foods available in this area. The second possible cause is the demographic characteristics of Taiwanese macaques. Hsu and Lin (2001) reported the population growth rate of Taiwanese macaques as 1.187, 1.244 and 1.290, considerably higher than the reported maximal rate for Japanese macaques (1.17). In which case, the hybrids are maintaining the population growth rate characteristics of Taiwanese macaques and not Japanese macaques. These seem very high compared to those of Japanese macaques. Increase of the population growth rate in the new favorable environment (Japan) is also the possible cause, but there is no data to support this idea. The third possible cause is heterosis or hybrid vigor, although there is no proof that this occurs in macaques. The first cause, a rich environment,

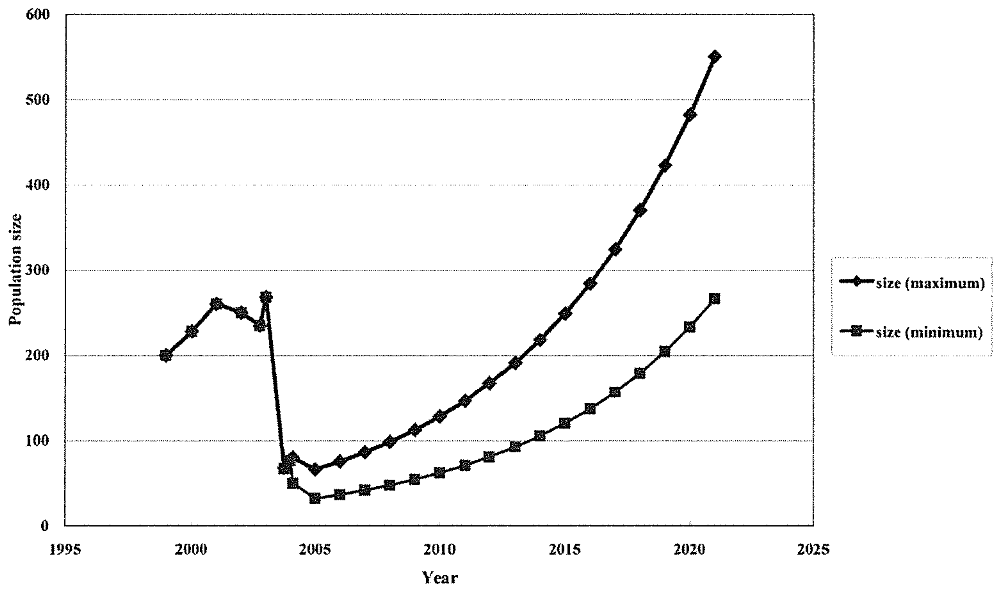

FIGURE 2. Estimated Population sizes of hybrid Taiwanese / Japanese macaques in Wakayama up to 2021 if the current capture program is abandoned, assuming no density dependent influences upon the population growth rate.

is the most likely, although the accuracy of the estimates of population size remains to be confirmed.

Counter measures to population growth

Since the rediscovery of the population of Taiwanese macaques in Wakayama, the Wakayama prefecture decided on a plan to eliminate this hybrid population with the aid of the ministry of Environment, Japan. The total number of eliminated animals since the first census (initial population size of 200) in 1999 reached 295 in 2004 (Table 1) and 329 in July 2005 (not shown). The plan of elimination was supposed to be nearly successful. Unfortunately, although Wakayama prefecture is attempting to continue the plan, it is hindered by financial difficulties. If this plan is stopped, the hybrid population will soon recover its numbers, as potentially shown in Fig. 1 and 2, and all the efforts until now will have been wasted. We, the working Group of Taiwanese macaques in Wakayama, are asking the help of the ministry of Environment and are also willing to support to continue this plan.

Comments on the rapid population growth in provisioned populations, newly introduced population or newly hybridized populations.

We present a case of initial population explosion in an introduced foreign macaque species in a rich environment. We also showed the rapid population growth in a provisioned population. When a group becomes enormous under unnatural conditions, the population must be controlled by means of elimination of animals to avoid disturbances of the ecosystem. Elimination of many animals can cause other problems, such as the heavy financial cost of mass killing. Transfer to other areas may result in another disturbance of a new environment or of the primate fauna. In South Asia there are many provisioned populations of monkeys. We need to manage them on the bases of conservation biology. In Japan there used to be many provisioned populations of Japanese macaques, however, now their numbers are decreasing.

Some cases of hybridization among macaque's species have been (Fooden 2000; Hamada et al., 2004; Malaivijitnond, S. et al.,

2004) as well as other unpublished observations (Suchinda and Ohsawa 2004, Wat Tham Kham, Sakhon Nakhon Province, North-Eastern Thailand). Such populations should be continuously monitored, because they may have a different niche from the original species, show unexpected population movement and adversely impact on the environment and the primate fauna.

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Ecological Diversity and Conservation of Japanese Macaques

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ABSTRACT.– **Most macaques in Southeast Asia are distributed over wide geographic ranges. Intensive studies on the ecology of the Japanese macaque, which is distributed over a great variety of habitats and displays considerable variations in behavior and ecology, indicate that we should carry out research on Southeast Asian macaques under various ecological settings in order to determine the general ecological features of a given species and plan the most appropriate conservation measures.**

KEY WORDS: *Macaca fuscata,* Distribution, Ecological variation, Adaptability, Conflict between monkeys and humans

INTRODUCTION

The Japanese macaque (*Macaca fuscata*) is a species endemic to the Japanese Archipelago that occupies the most northern frontier in the distribution of non-human primates. The distribution of this species ranges from 30°30' N to 41°31' N in latitude and covers a great variety of habitat types in subtropical, warm temperate and cool temperate climatic zones. Like Japanese macaques, most of the Southeast Asian macaques are distributed over wide geographic ranges, so studies on ecological variation in Japanese macaques could well provide guidelines on both conservation and how to study the ecology of such wide-ranging Southeast Asian species. Here, I briefly summarize the results obtained from ecological studies on Japanese macaques.

ECOLOGICAL VARIATION IN JAPANESE MACAQUES

The distribution of Japanese macaques is correlated with the area of broadleaved forests, except in northern Japan where high hunting pressure had been imposed on monkeys until 1947, and where the area currently devoid of monkeys is still substantial, despite the wide expanse of broadleaved forest (Koganezawa, 1991a). This implies that the deterioration of broadleaved forests & hunting have the potential to seriously threaten the survival of this species.

Japanese macaques consume mainly plant parts but the food plant species change with locality according to the type of vegetation available. Based on the meteorological data of precipitation and solar radiation, Uchijima and Seino (1985) estimated the net primary productivity of natural vegetations in each region of Japan. The maximum difference in productivity between the regions was found to be as great as 1.8 times. Plant productivity might determine the food availability for monkeys and food availability is considered to regulate not only population density but also troop sizes (Yamagiwa and Hill 1998). Thus, local differences of the plant productivity

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produce the local differences in such population parameters. Troop fission of wild Japanese macaques tended to occurs when the troop expanded to more than 70 individuals in deciduous broadleaved forests and 50 individuals in evergreen broadleaved forests. Other population parameters also differ with locality. In northern Japan, the severity of winter influences the mortality rate of infants and the birth rate far more so than in southern Japan (Koganezawa, 1991b).

The pattern of home range use also differs with locality. For example, in the deciduous forests of northern Japan, monkeys mainly feed on bark and buds in winter and they tend to move less. However, in autumn they move rapidly and feed mainly on fruits and seeds (Suzuki, 1965). This seasonal change in activity reflects the fact that a good supply of high quality food can compensate for the high energy expenditure of rapid and long-distance travel to obtain the food in autumn, whereas the animals tend to save energy in winter by moving less because only low quality foods are available and the cold weather saps the animals' energy. As winter food conditions in evergreen forests do not degrade as much as those in deciduous forests (Nakagawa et al., 1996), the seasonal change in home range use might not be as drastic.

Social relationships between troops are also influenced by local environment. For example, the difference in the rate of aggressive encounters between troops observed in two different areas were interpreted to reflect differences in the amount and the distribution of food resources in those areas (Saito et al., 1998).

CONFLICT BETWEEN MACAQUES AND HUMANS

Currently, Japanese macaques are faced with a serious problem because of their wide ecological adaptability. This problem has arisen not because of hunting pressure or habitat deterioration but because of conflicts with humans, such as the agricultural damage caused by monkeys and problems caused by monkeys invading human settlements. Their habitats are now expanding from forest areas into agricultural areas and human settlements. This habitat expansion or shift reflects both alterations in forest management policy and the lifestyle of humans. Furthermore, the provisioning of monkeys for tourism and scientific research have worsened the situation by accelerating the habituation of monkeys to humans and increasing the number of such habituated animals. Consequently, provisioning has been abandoned in most areas.

In addition to the damage done to human property by monkeys, excessive contact between humans and wild animals will increase the risk of transmission of infectious diseases.

People and governments are struggling to exclude monkeys from farmlands and human settlements and make them go back to forests, which represent 70% of the land area of Japan.

IMPLICATIONS FOR ECOLOGICAL STUDIES OF MACAQUES IN SOUTHEAST ASIA

Japanese macaques are considered to have deployed from the continent to the archipelago 0.6-0.4 million years ago. They experienced dramatic alterations in climate, vegetation, and geography during the glacial epoch. It has been suggested that extraordinary range of adaptability that the animals show at present, even to disturbed and fluctuating environments, might have been acquired through their evolutionary history during the glacial epoch (Richard et al., 1989). Although conclusions on the general features of the ecology of Japanese macaques are still contentious, the detailed ecological studies performed so far suggest that we should similarly carry out research on macaques in Southeast Asia under various ecological settings in order to delineate precisely their general ecological features and plan the most appropriate conservation measures for each species.

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Welfare for Primates as Research Resources

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ABSTRACT.– **Animal welfare is one of the most important concerns in the world for researchers using vertebrate animals. Among vertebrates, primates especially, are often given a special ethical status because of anthropomorphic sympathy for species of phenotypic / genotypic and behavioural similarity to humans, and because of their recognized complex cognitive abilities and psychological requirements. Researchers and caretakers of non-human primates have a duty to consider animal welfare in experimental procedures and husbandry conditions. It is strictly required to promote not only the physical well-being of primates but also the psychological well-being. The practical strategy to satisfy animals' psychological requirements is via environmental enrichment. People in charge of research with primates should always strive towards the improvement of experimental animals' welfare by enrichment of their captive environment on the basis of performance standards.**

KEY WORDS: Vertebrate animal welfare, Psychological well-being,
Environmental enrichment, Animal experiment

THE "FIVE FREEDOMS" AND WELFARE FOR ANIMALS

Animal welfare is one of the most important concerns in the world for researchers who conduct vertebrate animal (hereafter "animal" and thus excluding invertebrates) experiments. Experimental animals should receive good husbandry and welfare throughout their life, even if many of them are finally sacrificed. Non-human primates (hereafter primates) are often given a special ethical status. The basis for this special status for primates stems not just from emotive aspects in our ability to empathize most with our closest relatives and to anthropomorphize primates and / or their plight, but additionally from consideration of their recognized highly complex requirements, cognitive skills and the ability to feel pain and suffer. Their relatively larger brain to body size, sophisticated cognitive abilities and reliance on sociality and complex social systems lead us to believe that their perception on suffering as well as cognitive and social deprivation may not differ so much from ours.

Primates do not have specific rights that are different from other animals' ones. However, as outlined above, primates should be given specific consideration on animal welfare when they are used for research. That is, researchers and caretakers have a duty to ensure their animals live as comfortably as possible, adhering to the following "Five Freedoms" (UK Farm Animal Welfare Council, 1997):

1) Freedom from thirst, hunger, or malnutrition

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2) Freedom from discomfort
3) Freedom from pain, injury and disease
4) Freedom to express normal behaviour
5) Freedom from fear and distress

Although the "Five Freedoms" were advocated for farm animals, they should be much more rigorously applied to primates because of their perceived greater capacity for psychological suffering by isolation, boredom and restriction of behavior than farm animals. In addition, the Amendment to US Animal Welfare Act (1985) stated the requirement of "an appropriate plan for environmental enrichment adequate to promote the psychological well-being of non-human primates". In order to establish the welfare of primates we have to keep primates healthy not only in physical aspects but also in mental aspects.

Today, in accordance with this concept of primate welfare, many countries have active legislations to control primate husbandry and conditions for experimental use. Further, there are guidelines that show practical ways to ensure the appropriate husbandry and safeguard animal welfare. Singapore has *the Guidelines on the Care and Use of Animals for Scientific Purposes* produced by the National Advisory Committee for Laboratory Animal Research in 2004. The USA has *the Guide for the Care and Use of Laboratory Animals* produced by the National Research Council in 1996. In the UK, there are several codes, which provide general principles for the care and use of animals. Australia has the *Australian code of practice for the care and use of animals for scientific purposes* edited by National Health and Medical Research council in 1997. These guidelines show world-standards of physical and psychological requirements for welfare of primates, although the systems to govern the use and care of animals are different among countries.

CONSIDERATION OF EXPERIMENTAL PROCEDURE AND HUSBANDRY

When we consider animal welfare in research, there are two aspects that should be considered carefully. The first concerns experimental procedure, and the second concerns the condition of husbandry, that is the quality of life of experimental animals before, during and after the experiment. These two are explained in detail later. Legislations and/or guidelines of animal welfare, as shown above, require each institution to establish an ethical committee and institutional guidelines in order to regulate rearing and experimental uses of animals. In addition, in the UK both researchers and projects have to obtain personal and project licenses, respectively, from the Home office.

1: The"3Rs": consideration for experimental procedures

Given the legal and ethical barriers against the use of human subjects for experimental research, the use of other animals is important and essential to increase our understanding of animals from the biological and/or biomedical points of view. However, for the establishment of animal welfare, researchers always have to first consider alternative ways to pursue the aims of experiments. The concept of alternatives or the "Three Rs", that is, Reduction, Refinement, and Replacement of animal experiments was proposed by William M.S. Russell and Rex Burch in 1959. They reported the concept as the scientific study of humane techniques in animal experiments, commissioned by the Universities Federation for Animal Welfare.

Reduction alternatives are methods that use fewer animals to obtain the same amount of data, or that allow more information to be obtained from a given number of animals, and thus aims to decrease the total number of animals used in research. Therefore, researchers have to make efforts to plan more efficient experiments, to apply more precise statistic analysis to their data, and to reduce the number of animals used in the experiment by using ever-evolving cellular and molecular biological methods.

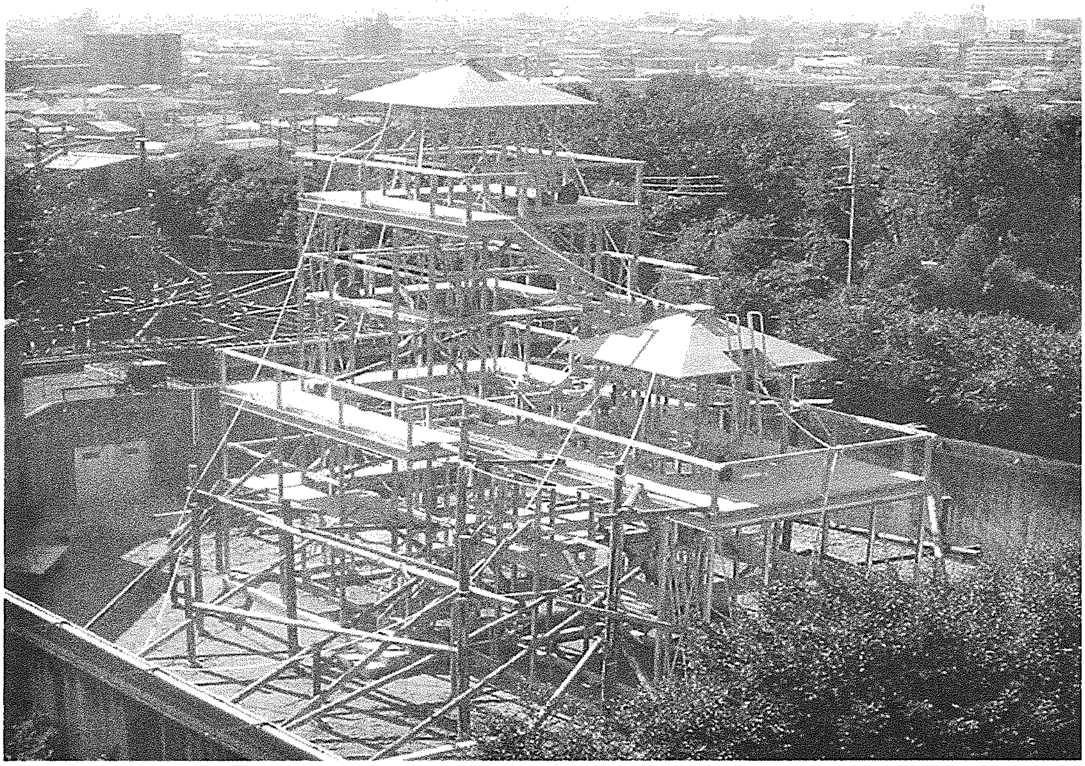

FIGURE 1. 3D construction named Frame-Kit Tower in a Japanese macaques' enclosure. This tower is 13m high. Animals spend more than 80% of the daytime in the space over 4m from the ground. Animals are distributed over several floors, so that they can each maintain a larger individual space.

Refinement alternatives are methods to minimize animals' pain and distress. It is indispensable to assess the intensity of pain each animal experiences. If we cannot find good objective measures of pain, it should be appropriate to assume that if the procedure is painful to human, it will also be painful to the proposed experimental animal. Although the original concept of the alternatives did not include the improvement of housing condition (e.g. environmental enrichment), it is now considered as an important aspect of the refinement alternatives (Bekoff and Meaney, 1998).

Replacement alternatives are methods that replace live animals with alternative experimental systems to attain the same data, such as the use of cultured tissues (*in vitro* systems) or by mathematical/computer simulations (*in silico* systems).

2: Physical and Psychological well-being

Animal welfare requires researchers involved in animal experiments to pay attention to the lives of the experimental animals before, during and after the experiment in addition to the experimental procedures. The welfare of primates will be maximized by keeping animals better in both physical and psychological well-being. First, in order to improve animals' well-being, it is necessary to prevent or minimize disease and injuries by assessment of physical health and by management of fighting injuries or nutritional imbalances. Second, it is necessary to satisfy animals' requirements. Animals' physical requirements cannot be separated easily from psychological ones. That is, when physical requirements are not satisfied in an animal, psychological ones are also not satisfied. Psychological well-being might be influenced by individual specific requirements

FIGURE 2.1 and 2.2. Foraging condition is important for feeding enrichment. Provision of food through moveable feeders (Figure 2.2), as opposed to free found food (Figure 2.1) is a useful device to lengthen feeding time and reduce boring time in captivity. (photographs by courtesy of Viktor Reinhardt: http://www.awionline.org/lab_animals/rhesus/Photo.htm)

that are determined on the basis of its species, sex and life histories, and can be assessed by the following criteria (National Research Council, 1998).

1) The animal's ability to cope effectively with change in its social and physical environment.

2) The animal's ability to engage in beneficial species typical activities.

3) The absence of maladaptive or pathological behavior (self-injury, stereotypy, etc.)

4) The presence of a balanced temperament and absence of chronic signs of distress (distress vocalization, posture, abnormal hormonal concentrations).

The practical strategy to promote psychological well being is the application of environmental enrichment that continually changes, and presents novel, appropriate and stimulating challenges to animals. By the introduction of environmental enrichment, animals will not passively receive the environmental events but actively work on the environment.

ENVIRONMENTAL ENRICHMENT

Primates have high cognitive ability and complex requirements to their environment, as mentioned above. It means that they are particularly prone to suffer from boredom when they are placed in barren and unstimulating environments. Enrichment of the environment increases the functional challenges animals cope with. Environmental enrichment attempts to elicit a species-typical activity pattern that includes species-specific behavioral repertoire and time budgets. This also means that environmental enrichment increases normal activities and reduces abnormal activities.

Environmental improvements by enrichment include opportunities for animals to engage in activities such as social interaction, exercise, foraging, and manipulation of objects in

FIGURE 3. Good relationships between humans and animals can elicit cooperation of animals in procedures such as venipuncture and injection. This relationship can also reduce distress and suffering caused by experimental treatment and/or husbandry.

addition to enrichment of the physical environment as space size and complexity, and cage furniture (perches, swings, ropes etc.). In addition, the improvement of the quality of interaction between animals and people might work as environmental enrichment as well, because it gives a chance of inter-species social interactions. Good relations between animals and the human(s) in charge of them might positively affect the research procedures, because it induces animals' cooperation and so reduces its distress in research. However, environmental enrichment does not always have beneficial results to research, even though it is always good for animals, and so the two must be considered and balanced. For example social housing might increase injuries and distress by social hierarchy, whilst a complex environment might be stimulating and initiate realistic interactions between individuals but may cause difficulties in the control of the animals. These seem to decrease the well being of animals and to affect negatively some research data such as hormonal concentration. Consequently, it might be impossible to say simply that environmental enrichment should be applied to experimental animals. It is necessary to refine the practical methods for environmental enrichment such as social housing with less conflict. In addition, we must remain open to the possibility that individual housing is the only way to carry out a particular study successfully. Even so, there cannot be a reason for us to refuse environmental enrichment for experimental animals. Researchers and caretakers should always try to improve the environment of experimental animals under the concept of "a bias for action" (Keeling et al., 1991). This states that researchers should do something for the animals as far as it does not harm the animals and / or the experimental conditions, even if its effect might not be proved sufficiently.

CONCLUSIONS

Consideration for animal welfare has become a global issue and movement today. People in charge of animal experiments cannot ignore the welfare of their animals, now that we have accumulated more and more knowledge about suffering, distress, and requirements of animals. It is essential to provide environmental enrichment in order to satisfy animals' mental requirements.

However, it is often not clear what specific benefits any given enrichment provides or which device(s) provide enrichment, inasmuch as there are various individual- and species-specific differences in responses to any given change. We can easily assess the attainment of environmental enrichment on the basis of physical parameters such as cage size. However whilst it might be often assumed that a larger cage would promote psychological well being, this may not always be the case. A previous study on the effect of cage expansion in primates did not conclusively report an improved animal welfare; e.g. reduction of abnormal behavior (Line et al., 1990, Crockett et al., 2000). That is, engineering standards, which focus on easily measured aspects of the physical environment or on attainment of minimum requirements, is not enough to assess psychological well-being. On the other hand, we have performance standards which focus on animals themselves and on their reactions to various features of life in captivity. Performance standards measure, for example, preference of objects and/or environments, intensity of signs of chronic distress as manifested in maladaptive or pathological behavior, and activity patterns altered by environmental enrichment. Consequently, in order to improve the welfare of animals, we should preferentially employ performance rather than engineering standards.

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Mitochondrial DNA Sequence Variation and Subspecific Taxonomy in the White-handed Gibbon, *Hylobates lar*

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ABSTRACT.- Noninvasive genotyping was employed to see if the five allopatric subspecies of *Hylobates lar* gibbons could be recognized as genetically differentiated evolutionarily significant units. Phylogenetic and haplotype network analyses were used to study variation of mtDNA control region sequences among 46 allegedly unrelated *H. lar gibbons* in North American zoos. Although the origins of these gibbons or their parents are unknown, they are the result of multiple independent importations, and it is likely that they include representatives of several subspecies. The export of *Hylobates lar* gibbons from Asia involved primarily animals referable to the northern (*carpenteri*) and central/southern (*entelloides*) subspecies described from Thailand. The mtDNA control region was selected for study as it is unusually variable and phylogenetically informative in most mammals. In this sample of *H. lar*, however, all individuals studied had sequences very similar to one another and to the two published complete mtDNA sequences for this species. A phylogenetic tree analysis provided only weak evidence of variation and no support for the presence of genetically distinct subspecies in this sample. In contrast, the haplotype network analyses showed more variability and indicated that this sequence may be informative enough to resolve phylogeographic patterns in this species. This can now be tested by genotyping *H. lar* gibbons of known geographic provenance.

KEY WORDS: Gibbons, mtDNA control region, Noninvasive genotyping, Subspecies identification, Thailand

INTRODUCTION

The recognition of subspecies to describe geographic variation in wide-ranging primates continues despite general appreciation that such taxa, as traditionally defined, may have little merit in identifying units of evolutionary significance (Wilson and Brown, 1953). Nevertheless, the needs of conservation managers to sustain innate local geographic variability justifies the search for genetic, morphological and behavioral markers of regional variability (Frankham et al., 2002). Furthermore, relatively new methods of phylogenetics provide us with the tools to characterize the genetic structure of surviving populations of increasingly fragmented and threatened primate species. We present an example of this approach with a preliminary study of *Hylobates lar* (Linnaeus), the white handed gibbon found historically across most of Thailand (Lekagul and McNeely, 1988).

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Hylobates lar gibbons are found in six countries in southeast Asia, ranging from Yunnan, China, in the north, south through Myanmar, Laos and Thailand to peninsular Malaysia and Sumatra, Indonesia. Today the populations on Sumatra are isolated from those on the continental mainland by water and those in peninsular Malaysia are isolated from those to the north by the presence of a congener, *H. agilis*, on the border of Malaysia and Thailand. The species range thus spans 2,200 km (22° latitude) and, not surprisingly, the extreme populations are morphologically different from one another. Local morphological variants have been used to describe five currently recognized subspecies (Corbet and Hill, 1992; Groves, 2001; Brandon-Jones et al. 2004; Geissmann, 2005).

Hylobates lar lar	Peninsular Malaysia, S. of Perak river
H. l. carpenteri	Montane northern Thailand, N of Phitsanulok; adjacent areas of eastern Myanmar, western Laos
H. l. entelloides	Western, central (including Khao Yai) and southern Thailand, adjacent areas of Myanmar
H. l. vestitus	Northern Sumatra, N. of Lake Toba
H. l. yunnanensis	Southwest Yunnan

We do not propose to review the allegedly defining characteristics of these subspecific taxa here but merely note that the morphological criteria employed in the past are not diagnostic even for adults, and fail to capture local and regional population variability. The utility of the alleged hair length and pelage differences used to distinguish the two Thai subspecies *carpenteri* and *entelloides*, for example, have been rejected by several workers (see Lekagul and McNeely, 1988), and Geissmann (2005) hypothesizes that *yunnanensis* is a synonym of *carpenteri*. As direct tests of the validity of these taxa are not yet possible, we will instead ask whether, in a large sample of *lar* gibbons of diverse origin, one can discern clusters of related individuals and, in turn, define geographically-based evolutionarily significant units.

This preliminary study takes advantage of our introduction of methods of noninvasive genotyping based on plucked or shed hair and the availability of powerful new statistical methods of phylogenetic analysis. Interestingly, the first demonstration that DNA could be amplified from the hair of a non-human primate (or any other non-human mammal for that matter) involved a pilot study of *H. lar* from the Dusit Zoo, Bangkok. Woodruff (1990: Figure 1A) demonstrated individual variability at a nuclear microsatellite locus in four *H. lar* individuals of unknown origins. Garza and Woodruff (1992) then reported mitochondrial (mtDNA) cytochrome b variation in seven *H. lar* but found only 0-3 transition and no transversion differences between four gibbons from Chiang Mai Zoo (in the range of *H. l. carpenteri*) and two from the range of *H. l. entelloides*. Subsequently, Monda (1995) studied a more variable mtDNA locus in a larger sample of *H. lar* and her unpublished control region sequence data form the basis of the present analysis. Several other researchers have prepared partial molecular phylogenies to study interspecific and intergeneric differences in the Hylobatidae but none have included more than 1-3 *H. lar* gibbons in their surveys and been able to investigate intraspecific variation (Hall et al., 1998; Zehr, 1999; Roos and Geissmann, 2001; Takacs et al., 2005; Chatterjee, 2006).

The noninvasive genotyping approach developed by the first author and his associates is described in detail elsewhere (Woodruff, 1993, 2003; Morin and Woodruff, 1996), and its power in characterizing and partitioning geographic variation is well-illustrated in our studies of species and subspecies of, for example, chimpanzees (Morin et al. 1994; Gagneux et al., 1999), African elephants (Eggert et al., 2002) and birds (Mundy et al., 1997; Eggert et al., 2004).

Collecting DNA for the noninvasive genotyping of wild gibbons is extremely slow work and although several researchers are collecting fecal and urine samples few results have yet been published (Lappan, 2004; Whittaker et al., 2004). In the interim, in an attempt to find a useful marker sequence we conducted a survey of variation in captive animals in 49 North American zoos. We examined 79 *H. lar* gibbons of which 46 were thought to be unrelated. All were of unknown origin except for three identified by their owners as *H. l. carpenteri* and one as *H. l. entelloides*. As most of these gibbons or their parents had independent importations from Asia, we assumed that we might encounter gibbons shipped from Thailand, Malaysia, and Indonesia. We therefore expected to sample individuals that, had their ancestral provenance been known, would be referable to 2, 3 or 4 of the recognized subspecies. The goal of our survey was to determine if the mtDNA control region was sufficiently variable to be useful in studies of phylogenetics at generic, species and subspecies levels; herein, we focus exclusively on the case of intraspecific variation in the species *H. lar*.

MATERIAL AND METHODS

Samples

In cooperation with the American Association of Zoological Parks and Aquariums (AZA) Gibbon Taxon Advisory Group (TAG), plucked hair samples from 79 *Hylobates lar* individuals were sampled from 49 North American zoos. Forty six of these gibbons were allegedly unrelated and are used in this analysis. These individuals are identified elsewhere (Monda, 1995; Simmons, 2005; Woodruff et al., in prep.). Taxonomic identifications were provided by the various holding zoos: one animal was identified as *H. l. entelloides*, two as *H. l. carpenteri*, and 44 as "subspecies unknown". For each individual, DNA was extracted from hairs and the mtDNA control region was amplified and sequenced by Monda (1995). We also determined sequences from *H. agilis* and *Symphalangus syndactylus* (the siamang) for use as outgroups in various analyses. Finally, two complete *H. lar* mitochondrial sequences (Genbank: NC002082 and X99256), a *H. lar* d-loop sequence (Roos and Geissmann, 2001: AF3111723) and a published gibbon numt pseudogene sequence (AF035467; numt: the abbreviation for a nuclear sequence of mitochondrial origin) were used to verify the mitochondrial origin of our target sequence, and in an attempt to capture additional variability.

Extraction, sequencing and sequence verification

Roots of one to three individual hairs from a single animal were digested in 5% Chelex resin (BioRad). Initially, a 500 bp segment of the mitochondrial genome control region was PCR amplified using two oligonucleotide primers, L15997 and H16498, identified according to the human nucleotide reference sequence (Anderson et al., 1981). Procedural details for amplification, electrophoresis, and direct sequencing using ^{32}S labeled nucleotides are given elsewhere (Monda, 1995). Simmons (2005) subsequently verified Monda's sequence data by re-extracting and re-sequencing DNA from archived hair samples from some of the same individuals. To reduce the risk of possible contamination, the verifying extractions, PCR, and sequencing were performed in a laboratory in which non-human primate DNA had not previously been handled. We used the DNEasy extraction kit (Qiagen) and re-amplified 325 bp from the control region using the primers H16431 (Kressirer, 1993) and L16007 (Roos and Geissmann, 2001). Sequencing of both strands of DNA was performed on a 3100 Genetic Analyzer using Sequencing Analysis and Data Collection software version 5.1 (Applied Biosystems), and verified with Sequencher Version 4.2 (Gene Codes Corporation). Sequence data (and Genbank numbers, when assigned) are available from the senior author.

FIGURE 1. Distance phylogenetic tree based on mtDNA control region sequence variation in *Hylobates lar* gibbons. Outgroups are *Hylobates agilis* and *Symphalangus syndactylus*. Three gibbons reported by their owners as belonging to subspecies *carpenteri* (C) and *entelloides* (E) are identified; all others are of unknown subspecific identity. Individual identities of all animals are available from the authors.

Data Analyses

We aligned the sequences using Clustal X Version 1.81 (Thompson et al., 1997) and verified the alignments by eye. We constructed a phylogenetic distance tree using PAUP* Version 4.0b (Swofford, 1998); rooted by outgroup as shown in Fig. 1. Intraspecific gene genealogies were inferred using the method of statistical parsimony, TCS (Clement et al., 2000), to calculate the probability of parsimony for all haplotype pairwise differences until the probability exceeded 95%. The program generates a network linking closely related haplotypes by the maximum number of mutational differences or steps and leaves all other haplotypes as outgroups.

RESULTS

A phylogenetic distance tree is shown in Figure 1. Almost no differentiation is seen among these 46 *H. lar* gibbons, although their haplotypes are very different from the outgroups, *H. agilis* and *S. syndactylus*. This result was consistent across trees prepared with parsimony, maximum likelihood, and Bayesian methods (Simmons, 2005). Two of the subspecifically identified gibbons lie at the top of the tree: the alleged *entelloides* (E) lies in the same clade as an alleged *carpenteri* (C). The second *carpenteri* (C) lies in the middle of the tree apparently indistinguishable from three other gibbons. The lower portion of the tree groups 10 gibbons of unknown origin in a clade (herein termed Clade B) and below that two individuals appear as outliers. If the three gibbons identified to subspecies by their owners are correctly identified, then the phylogenetic trees show no distinction between carpenteri and entelloides.

The statistical parsimony network (Figure 2) shows a quite different pattern with far more variation than the phylogenetic trees. There are two large complex and loosely linked clusters of 35 haplotypes. 12 haplotypes lie in the upper subcluster, 22 lie in the lower subcluster, and one (in the top left of the lower subcluster) is of ambiguous affinity. Each haplotype was found in from one to five individuals in our sample. The outgroups, *H. agilis* and *S. syndactylus* lie independently outside the cluster, as expected, and all but one of the *lar* gibbons lie in this complex net of relationships. The isolated *lar* individual (AZA Studbook No. 716, a captive born female of unknown geographic origin), is not necessarily unrelated to the others, it simply violated the 5% probability parsimony criterion or limit. The haplotype with the highest outgroup probability is displayed as a rectangle (represented here by three individual gibbons and the two published whole *lar* mtDNA sequences), the other haplotypes are displayed as ovals. The two alleged *carpenteri* haplotypes (labeled C in Fig. 2) are positioned separately, one in each subcluster. One lies two mutational steps from the whole mitochondrial genome haplotype, the other nine steps. The alleged *entelloides* shares a haplotype (labeled E) with another individual and lies two mutational steps away from the whole mitochondrial haplotype. As in the previous analysis we cannot distinguish these two subspecies.

DISCUSSION

In view of the lack of reliable morphological subspecies identification criteria and the lack of information regarding geographic provenance of zoo animals, all identifications associated with the gibbons studied must be regarded as hypothetical. There is no corroborating information proving the subspecies identity of the few individuals identified as carpenteri or entelloides. This unfortunately precludes resolution of the taxonomic issues under discussion. However, in the absence of such verifiable identifications, we were at least able to analyze our results without taxonomic bias.

Initially, we feared that the lack of variation seen in the distance tree (Fig. 1) was due to our inadvertent sequencing of a molecular fossil, a nuclear pseudogene of mitochondrial origin or numt. We reject this hypothesis as the known numt in gibbons lies well outside the outgroup shown on the tree presented here (see also Simmons et al., in prep.), and as the two complete *H. lar* mtDNA sequences lie well within our cluster. We conclude that plucked hair is a replicable source of reliable control region sequence data.

The apparent lack of variation seen in Fig. 1 might arise if *H. lar* is a recent species and has rapidly expanded its range from a small base. Alternatively, this result could arise if the species has recently experienced a significant loss of variability as a result of a selective sweep associated with a new pathogen, etc. The taxonomic consequence of this result, if it were possible to identify the point of origin of each gibbon and if mtDNA control region variation were the sole criteria, is that the subspecies included in this sample are indistinguishable. Such a conclusion is premature, however, as

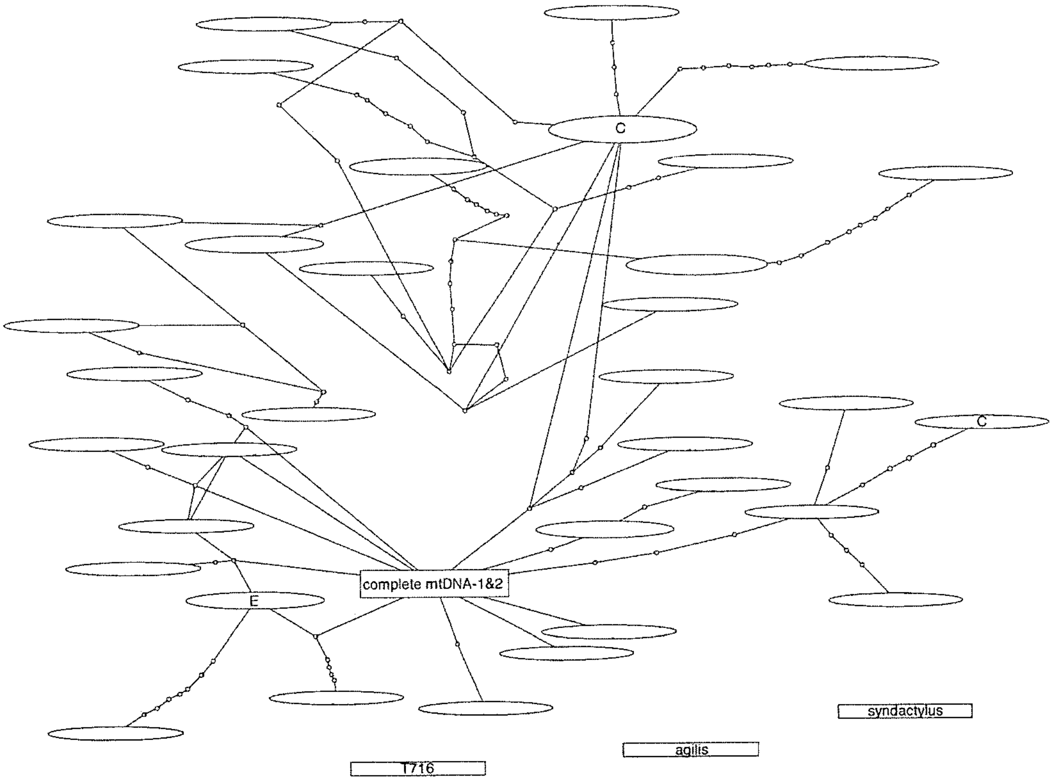

FIGURE 2. Statistical parsimony haplotype network of *Hylobates lar* individuals shown in Fig. 1. The large rectangle in the lower center represents the modal haplotype identified by the algorithm (represented here by three gibbons and the two published whole mtDNA sequences) and the ovals represent derived haplotypes. Dots between the known haplotypes indicate the number of hypothetical single-base pair mutational changes. Unconnected rectangles below the network represent outgroups (*Hylobates agilis, Symphalangus syndactylus*) and a single *H. lar* found to be less related to one another and the other gibbons.

the network analysis suggests that there may in fact be sufficient variation to describe geographic patterns.

It is possible that our sampling represents only one subspecies, and consequently it makes sense that the phylogenetic tree analyses fail to show clusters of unrelated individuals (although we did note a weakly defined Clade B and two outliers in Fig. 1, and one outlier in Fig. 2). Although much of the gibbon export trade may have been through Bangkok in the 1980's and earlier, it is most unlikely that all these animals in North American were of Thai origin. Juvenile gibbons were moved as pets and trafficked all over southeast and east Asia until quite recently. Even if Bangkok was the port of origin of all North American *H. lar* gibbons, it is most unlikely that all these animals, or their wild-caught parents, lived nearby, in the historical range of *entelloides*. Most gibbons abandoned by their owners at Bangkok's Dusit Zoo, or confiscated and brought to that zoo, were probably acquired as juveniles far from Bangkok.

The haplotype network analysis does in fact show more variation in *H. lar* than suggested by the phylogenetic trees. We hypothesize that there is enough variation between the 36 haplotypes discovered to eventually discriminate between gibbons from different parts of the species range. Whether the currently recognized subspecies are genetically distinct evolutionarily significant units can now be tested with comparable sequences from a few animals of known provenance.

Despite the inconclusive nature of this study, the patterns revealed by the haplotype networks are encouraging as they suggest that further work with the control region sequence may resolve the issues concerning geographic variation in *H. lar* gibbons. There is also some prospect for being able to sort the captive *H. lar* gibbons into geographically defined clusters so as to improve the captive breeding program and ultimately plan appropriate reintroduction experiments (Brockelman, 1994; Woodruff and Tilson, 1994).

Had the haplotype network patterns not shown this variability we would have advocated shifting attention to nDNA microsatellite loci. Microsatellites are an order of magnitude more variable than the control region so there is little doubt that they would reveal the innate phylogeographic pattern (Morin and Goldberg, 2003). However, such an investigation would be much more expensive, as a suite of 7-10 loci/individual would have to be genotyped, as nuclear sequences are harder to obtain from hair, feces and urine samples, and as many more individuals will have to be genotyped. We conclude, therefore, that additional surveys of control region variation are worth pursuing.

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Using Long-tailed Macaques (*Macaca fascicularis*) as a Model for Osteoporosis Study

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ABSTRACT.- **Ovariectomized female long-tailed macaques were used as a human model for osteoporosis. Each animal was measured for total body characteristics and lumbar vertebrae characteristics by dual energy X-ray absorptiometory, and measured for biochemical markers of bone resorption and formation. The measurements showed that bone mineral content for the total body and for the lumbar vertebrae, and bone mineral density of lumbar vertebrae were significantly decreased after the ovariectomy. Although no changes were detected in the serum concentrations of the biochemical markers of bone resorption, significant increases were detected in the serum concentrations of the makers of bone formation. The process of loss of bone mass after ovariectomy is analyzed.**

KEY WORDS: Long-tailed macaque, Osteoporosis, Ovariectomy, Bone formation marker, Bone resorption marker

INTRODUCTION

Nonhuman primates are widely used in bone research due to similarities in their reproductive functions and skeletal systems to humans (Yoshida, 1999), including reproductive endocrinology (menarche, regular menstruation and spontaneous menopause), increased bone turnover and bone loss in association with estrogen depletion, and skeletal micro architecture as revealed by histomorphometry. Osteoporosis is one of the most common diseases in the aged and constitutes a significant public health problem in the world. Ovarian hormone deficiency is considered the major risk factor for osteoporosis. Several animal species have been used in studies on loss of bone mass, including rat, mice, and primates. Long-tailed macaques maintained at Tsukuba Primate Research Center have been used in several analyses of bone mass, bone gain with growth and bone loss with aging. The objective of this study was to clarify the process of bone loss after ovariectomy in matured female long-tailed macaques as the animal model of post-menopausal osteoporosis.

MATERIALS AND METHODS

Laboratory-bred long-tailed macaques (*Macaca fascicularis*) were used in this study. Fourteen animals were divided into two groups; ovariectomized (OVX) (seven animals, 14.3±1.8 years old); and control (seven animals, 15.1±2.5 years old). All animals were kept in individual cages in an air-conditioned room in which the temperature was maintained at 25 ± 2 °C, the relative humidity at 60 ± 5 %, and with artificial lighting between 0500-

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TABLE 1. Measurement items

Item		Abbreviation
Whole body	Body weight	BW
	Lean tissue mass	Lean
	Fat tissue mass	Fat
	(Fat tissue mass)/(Lean tissue mass+Fat tissue mass) x 100	%Fat
	Bone mineral content	BMC_{TB}
Lumbar vertebrae	Bone mineral content	BMC_{L}
	Bone area	Area_{L}
	Bone mineral density	BMD_{L}
Biochemical markers of bone turnover		
Formation marker	Intact osteocalcin (bone Gla-protein)	OC (BGP)
	Bone alkaline phosphatase	B-ALP
	Amino-terminal propeptide of type 1 procollagen	P1NP
	Carboxy-terminal propeptide of type 1 procollagen	P1CP
Resorption marker	Pyridine cross-linked carboxyterminal teleopeptide of type 1 collagen	1CTP
	Tartrate-resistant acid phosphatase	TrACP
	Pyridinoline	Pyr
	Deoxypyridinoline	D-Pyr

1900h daily. They were fed commercially prepared monkey food, apples and oranges. The breeding procedure and the rearing conditions were the same as previously described (Honjo, 1985). Previously described methods were used for the measurement of total body characteristics (Narita et al., 1994) and lumbar vertebrae characteristics (Hiyaoka et al., 1994) by dual energy X-ray absorptiometory (DXA, Lunar DPX-α).

Table 1 lists the parameters that were measured, including biochemical markers of bone metabolism. Serum samples were used for determination of biomarkers excepting pyridinolin and deoxypyridinolin. These two markers were determined using urinary samples and corrected using urinary creatinine concentration as a standard.

RESULTS

Measured values for the OVX group were compared with those for the control group. Although no change in body weight after ovariectomy was observed in the OVX group, a significant decrease in the bone mineral content of the total body was detected (Fig. 1). No changes were detected in the lumber vertebrae (areas L3-L5) in the OVX group. However, the bone mineral content slightly decreased and the bone mineral density significantly decreased (Fig. 2). In the OVX group 16 weeks or more after overiectomy, the serum concentrations of B-ALP were significantly higher than those in the control group (Fig. 3). Both the serum concentrations of other bone formation markers in the OVX group tend to show higher values than those in the control group. In contrast, bone resorption markers in serum and urine samples showed no significant difference in the concentration between in the OVX group and in the control group (Fig. 4).

DISCUSSION

Because of their many similarities to humans, long-tailed macaques are frequently used in research on bone diseases (Bowles et al., 1985. Miller et al., 1986). In humans, although the data do not always concur, probably because of the different subjects nationalities (assumed genetic differences) or analysis methods used, most studies show that linear growth of bones ceases at around the age of 20 years, but that the bones continue to mineralize until bone mass peaks in the third decade of life. In our study, female long-tailed macaques manifested similar changes. The menarche of female long-tailed macaques occurs at about 2.5 years old and menopause at about 22 years with an estimated lifespan of over 30 years (Yoshida, 1990). In our study the

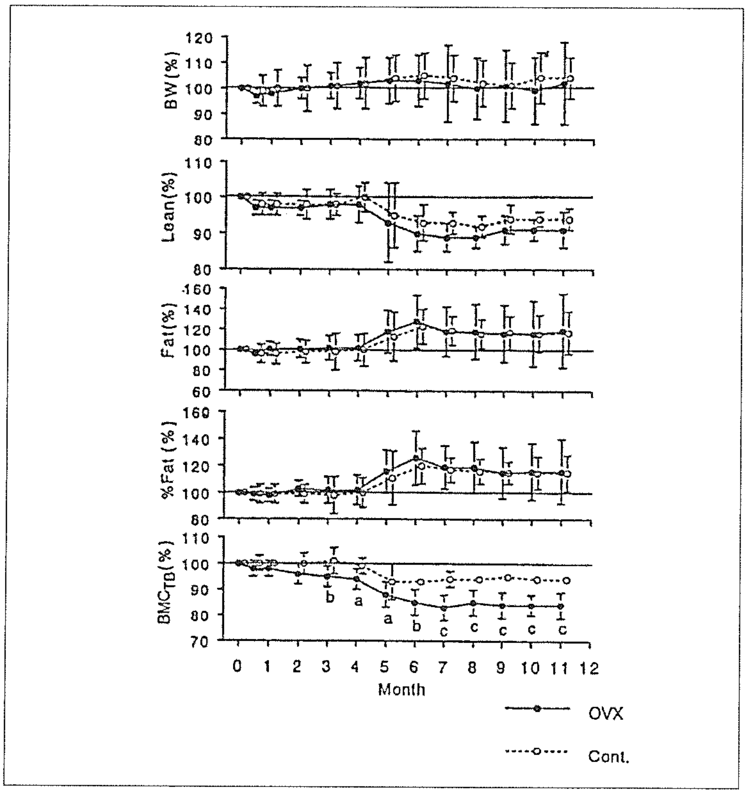

FIGURE 1. Measurements (Mean+SD) of whole body after ovariectomy in female cynomolgus monkeys (a; $p<0.05$, b; $p<0.01$, c; $p<0.001$).

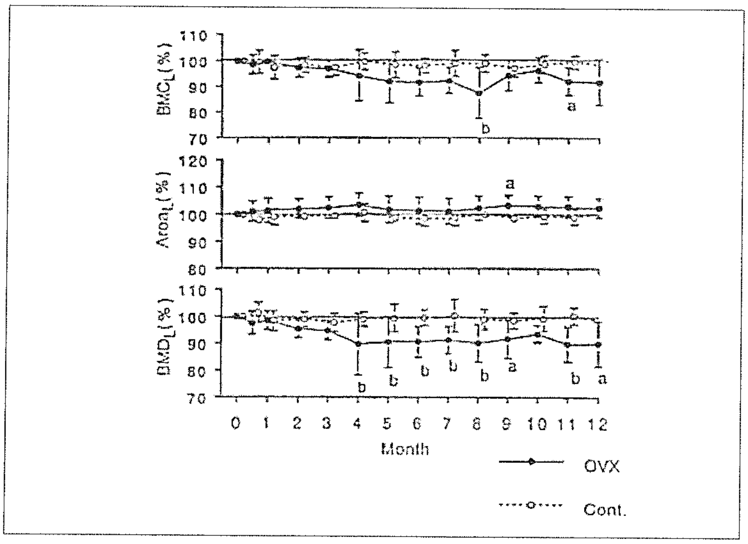

FIGURE 2. Measurements (Mean+SD) of lumbar vertebrae after ovariectomy in female cynomolgus monkeys (a; $p<0.05$, b; $p<0.01$).

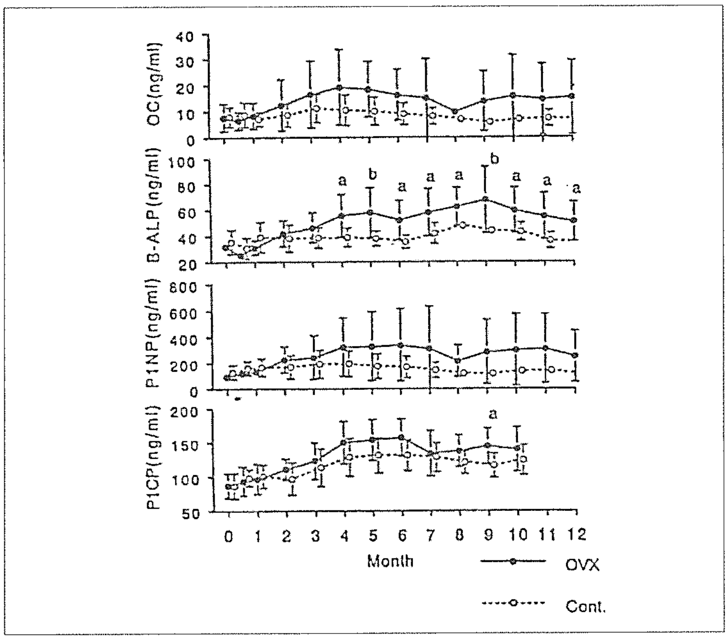

FIGURE 3. Measurements (Mean+SD) of serum bone formation markers after ovariectomy in female cynomolgus monkeys (a; p<0.05, b; p<0.01).

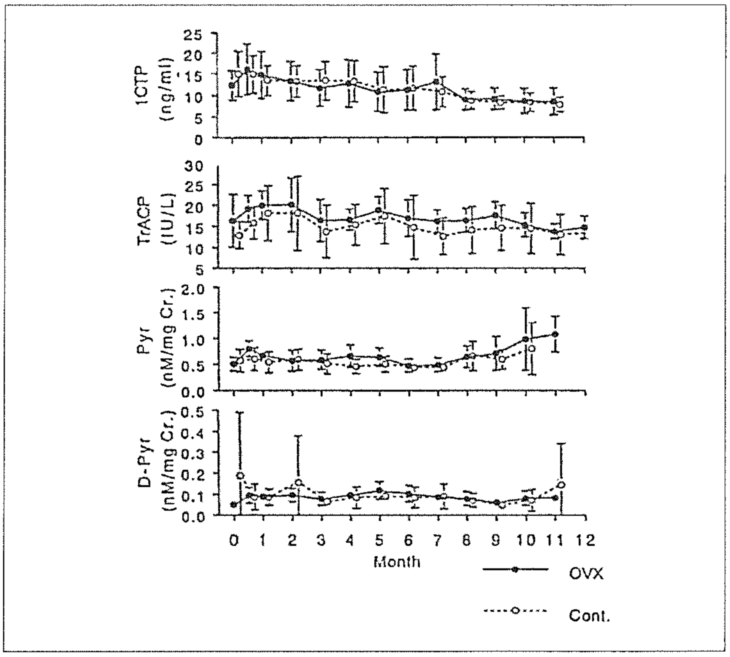

FIGURE 4. Measurements (Mean+SD) of serum urinary bone resorption markers after ovariectomy in female cynomolgus monkeys.

growth of the lumbar vertebrae was rapid during prepubecence, and then growth slowed after menarche. The bone mass peaked at about 9 years of age (Chen et al., 2000). Jayo et al. (1994) suggested that female long-tailed macaques achieve peak bone-mass at the age of 9, in agreement with our study, but they found that the bone mass tended to decrease in older mature animals of more than 13.5 years.

The significant reduction of the bone mineral content of the total body and the bone mineral density of the lumber vertebrae after ovariectomy were demonstrated. We suggest that the age of animals at ovariectomy is important to successfully induce the reduction of the bone mineral content and the bone mineral density after ovariectomy. Since we used animals over 9 years old in this study they had already achieved peak bone-mass.

Although serum concentrations of bone formation markers in the OVX group tend to show higher values than those in the control group, serum and urine concentrations of bone resorption markers showed no significant differences between two groups. This phenomenon may indicate accelerated bone formation with no change in bone resorption rates, but rather with a reduction in bone minerals. The reason for this discrepancy is not clear and further analyses are needed.

In conclusion, ovariectomized long-tailed macaques are judged to potentially be a suitable animal model for post-menopausal osteoporosis study.

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Short Note

Population Genetics and Its Application for Conservation with Examples of Japanese Macaques

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Japanese macaques, *Macaca fuscata*, are an endemic species of non-human primates in Japan and have the northernmost distribution of all non-human primate species in the world. In this presentation, I will review population genetic studies on this macaque species and will introduce recent application of molecular genetic markers to the management or conservation of macaque populations in Japan.

Early work of the population genetics of Japanese macaques was done by Profs. Nozawa and Shotake and their colleagues at the Primate Research Institute, Kyoto University (Nozawa et al., 1982, 1991). They examined multi-locus variation of blood proteins and studied genetic diversity within and between local populations. They found that protein alleles were not distributed uniformly across the entire range of the species and that genetic variability within groups was lower than in other macaque species. When differentiation among local populations was estimated as genetic distance, the result of clustering analysis showed significant deviation of the geographically peripheral populations. This geographic differrentiation was observed in the populations of Shimokita Peninsula in north, and Bousou Peninsula and Yakushima Island in south. From this result, Nozawa and his colleagues hypothesized that two or more waves of immigrants had colonized Japan (Nozawa et al., 1982, 1991). Examination of the correlation between protein genetic distance and geographic distance revealed that the gene constitutions of troops more than 100 km apart on an island could be regarded as independent of each other. Results of these protein studies suggest that the population structure of Japanese macaques involves a number of local subpopulations in which the effects of random genetic drift prevail. Nozawa et al. (1982) proposed a hierarchical model to account for this population structure combining a two-dimensional stepping stone model and an island model to explain frequent gene flow among neighboring groups and infrequent gene flow among local populations (local concentration of groups).

More recently, a population genetic study based on mitochondrial DNA (mtDNA) variation gave a different result from that of the previous protein studies regarding genetic diversity and differentiation among local populations (Kawamoto, 2002). The results of mtDNA study do not support the two wave migration hypothesis because observed mtDNA phylogeographic patterns can be explained as a result of monophyly with subsequent population expan-sion. The latter probably occurred after exten-sive extinction of the ancestral populations and/or disappearance of their habitat during the last glacial period in eastern Japan.

Molecular markers based on proteins and DNA are frequently used in recent genetic monitoring of macaque populations in Japan (Kawamoto et al., 1999, 2001, 2004, 2005). Molecular population genetic characterization provides basic information on history and structure of a populations (Avise, 1994). Obtained information is useful in making management plans for conservation of endangered local populations and for pest control of agricultural damages. Population genetic studies also become important for preventing adverse effects on ecosystems caused by invasive foreign species in Japan (Ministry of the Environment, 2004).

Japanese macaques are social animals, generally living in matrilineal groups consisting of multiple males and multiple females (e.g. Itani, 1954). Females remain in their natal group, while males usually tend to leave their birthplaces before full sexual maturity (e.g. Wada et al., 1974). This ecological feature of Japanese macaques produces characteristic distribution patterns of genes. Phylogeographical study of mtDNA, a maternally inherited marker, for females gives insights on historical background of local populations, and that for males gives evidences of interlocal migration through adult male transfer. The development of male specific genetic markers, such as Y-linked genes, is highly expected at present to evaluate isolation of endangered local populations. Meanwhile, autosomal markers are useful in assessing local inbreeding of natural populations and assortative mating in hybridizing populations. Population genetic study is particularly important at present in Japan in the genetic monitoring of introduced foreign macaque populations that have hybridized wit native Japanese macaques (e.g. Kawamoto, 2005).

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Gibbon Foraging Behaviour and Forest Ecology

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ABSTRACT.– **Gibbons (Family Hylobatidae) inhabit species-diverse evergreen forests and moist deciduous forests in South and Southeast Asia that provide a supply of fruits, shoots, flowers, and young leaves the year round. Gibbon groups (average 3–5 individuals) defend relatively small (10–30 ha) territories that contain sufficient food for the group in all seasons. The gibbon group on the forest dynamics plot at Mo Singto utilizes approximately half of the ca. 200 species of trees on the plot as well as many of the approximately 130 species of woody climbers (lianas) as food. Many of these fruiting species are dispersed by gibbons that swallow and defecate the seeds unharmed. Ecological research on the plot reveals that some fruiting species are dispersed almost exclusively by gibbons whereas most are potentially dispersed by a large variety of birds and mammals. Most mammals other than gibbons, however, are either seed predators or drop the seeds under the tree after consuming the flesh. Species dispersed primarily by gibbons tend to have relatively large seeds and covers or rinds that must be removed by the canines, and because they are dropped under the forest canopy, must have shade-tolerant seedlings. Such species, when ripe, are visited regularly by gibbons and their populations are likely to be limited by the capacity of the dispersal agent to consume fruit. Gibbon-dispersed species of trees and lianas are potentially in competition if their fruiting seasons overlap, but intraspecific competition for dispersal will be greater if their fruiting seasons are short. Therefore it can be predicted that gibbon-dispersed specialists will have staggered fruiting seasons and their diversity will be limited by fruiting season length. Species limited by their dispersal agents should be recruitment limited, meaning that density-dependent effects are not likely limit tree growth except under the parent tree. Such species could become threatened if their major dispersers are eliminated from the forest.**

KEY WORDS: Gibbon, Foraging behavior, Mo Singto

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Primate Diseases in Taiwan

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ABSTRACT.– We report on data derived as part of a continual health monitoring and disease surveillance of wild and zoo animals in Taiwan. Routine clinical observation and pathological examination of animals from several zoos located at northern and central Taiwan were performed. Two hundred and forty eight necropsy cases of non-human primates were collected in the past fourteen years (1991-2004). The pathological, microbiological, and electron microscopic examinations revealed that the major causes of death were bacteria (30.1%); parasite (20.5%); trauma and accidental death (16.9%); nutritional, metabolic, and immune-related disease (13.3%); unknown (10.2%); fungus (3.1%); tumor (2.9%); virus (1.5%); malformation (1.4%); and poisoning (1.4%). The major parasitic diseases included amoebiasis, anagiostrongylosis (*Angiostrongylus cantonensis* infection), enterobiasis (*Enterobius* spp. infection), oesophagostomiasis, toxoplasmosis, trichuriasis, *Physalopteridae* spp., and *Demodex* infection. Important bacterial diseases included mycobacterial, *Streptococal*, *Klebsiella*, and *Staphylococal* infection. Fungal disease included candidiasis, cryptococcosis and mucormycosis. This study also plays an essential role on the monitoring those potentially zoonotic diseases. The information will be beneficial for animal disease control and furthering the understanding of the zoonotic disease situation in Taiwan.

KEY WORDS: Primate; Zoonotic diseases; Amoebiasis

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Genetic Diversity and Phylogeography of Long-tailed Macaques in Southeast Asia

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ABSTRACT.– There are 19 species of macaque monkeys in the world and five of these species are recorded in Thailand, i.e. *Macaca mulatta*, *M. amensis*, *M. nemestrina*, *M. arctoides*, and *M. fascicularis*. *Macaca fascicularis* or long-tailed macaques are particularly of interest because they are widely distributed in Southeast Asia and encompass the Isthmus of Kra - an important biogeographic line that separates Indochinese and Sundaic genotypes for many faunas. Therefore we studied the molecular genetic diversity and phylogeography of long-tailed macaques in Thailand and other countries in Southeast Asia in order to infer an evolutionary scenario of the monkeys in this region. Blood samples were taken from long-tailed macaques temporarily trapped from 12 sites across Thailand and 4 sites from other countries. Total DNA was extracted and a partial D-loop region of the mitochondrial genome was PCR amplified and sequenced. D-loop DNA sequences of *Macaca sylvanus* and *Macaca mulatta* (rhesus monkeys) were also added to the data matrix before phylogenetic analysis. Phylogenetic trees were reconstructed using both distance and parsimony methods. We found a significant genetic difference between long-tailed macaques above and below the isthmus. Genotypes also clustered into smaller groups that co-mapped with their regional distribution. These findings reflect an influence of compartmentalization in long tailed macaque evolution in Thailand and Southeast Asia. Additionally, an ancient gene flow between long-tailed and rhesus macaques, probably in the interglacial or postglacial periods, are also postulated. Further phylogenetic studies, especially using other nuclear and mitochondrial gene sequences, of Southeast Asian long-tailed macaques are necessary to confirm these hypotheses.

KEY WORDS: Genetic diversity, Long-tailed macaques, *Macaca fascicularis*, Phylogeography, Southeast Asia

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Distribution and present status of long-tailed macaques (*Macaca fascicularis*) in Lombok Island (Indonesia)

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ABSTRACT. – **Lombok Island is a member of the lesser Sunda Island chain. It is situated east of Bali Island (8°30 S, 116°48' E at Mataram, the capital city of West Nusa Tenggara Province). Lombok has an area of 4,739 km^2 with Gunung (=Mt.) Rinjani as the highest mountain (3,726 m). The climate of Lombok is hot and drier than other neighboring regions (annual rainfall, 2000 ml). However, as especially evident on the higher mountains, the microclimate varies widely in the island, creating and supporting diverse habitats for flora and fauna. Although Lombok is situated east of the Wallace line, long-tailed macaques (*Macaca fascicularis*) have been distributed naturally there. They also live sympatrically with ebony leaf-monkeys (*Trachypithecus auratus*). According to a monograph written by Fooden (1995), long-tailed macaques were known from both Gunung Pengsong and Gunung Pusuk in the Suranadi area. Kawamoto *et al.* (1982) reported that long-tailed macaques were captured from Gunung Pengsong, Pusuk forest on the out -skirts of Gunung Rinjani. Since these reports, humans have significantly altered the habitat conditions . In 2001 - 2004 we conducted field surveys of long-tailed macaques in Lombok Island., From the seven places that were inhabited by troops of long-tailed macaques (Gunung Pengsong (temple), Lemor (forest), Suranadi (forest), Sambelia (village), Obel-Obel (forest), Pusuk (forest), and Rinjani National Park) we recorded 21 troops and a total of 335 individuals. Long-tailed macaques in Lombok reveal considerable adaptability as revealed by the diverse range of habitats colonized. Troops were found to live in lowland forest, sub-alpine forest, lakeside, savanna and temples. Some troops (Plawangan in Mt. Rinjani National Park) were found to live at high altitude (2,660 m), whilst other troops (Segara Anak in Mt. Rinjani National Park) were found to also live in a hot spring area (around Segara Anak Lake, 2,060 m). Some troops were provisioned; others remained wild and some are regarded as pests since they raid crops. The biodiversity of long-tailed macaques are considered to be decreasing (or being maintained at best). Currently, the principal threats on Lombok long-tailed macaques, together with leaf-monkeys, are deforestation and excessive hunting. An intensive survey of these primate populations is an essential prerequisite in order to take appropriate measures to preserve them in Lombok Island.**

KEY WORDS: Long-tailed macaques, Lombok island, High altitude macaques,
Hot spring

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The Implication of Malaria Studies in Macaques for Humans

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ABSTRACT.– Wild (non-human) primate populations are considered to be important sources for certain emerging infectious diseases in humans. Disease transmission can be unidirectional from non-human primates to humans or, importantly, bidirectional (occur in both directions). Non-human primates may be infected with the close relatives of important current human pathogens providing informative comparative models for both evolutionary studies of host-switching patterns and the evaluation of the potential origin of these pathogens including human malaria.

To date more than 26 species of *Plasmodium*, the causative agents of malaria, are known to circulate among primate populations. Of these, 4 species are known to be current human pathogens while at least 5 species belonging to non-human primate malaria have the potential to cross-transmit to humans under experimental, accidental or natural infections. Erroneously ignoring the above points, the role of non-human primate malaria in human disease was previously dismissed as relatively unimportant due to the fact that infected human cases were rare in nature. However, recent studies have revealed that *Plasmodium knowlesi*, a malaria species found in *Macaca fascicularis* in Southeast Asia, is responsible for illness in more than half of malaria infected patients in a community in Malaysian Borneo. In addition, identification of a Thai patient who naturally acquired *P. knowlesi* infection from a forest in Prachuap Khiri Khan Province in southern Thailand has substantiated the possibility that more cases would be encountered. This is because *P. knowlesi* resembles *P. malariae* both in terms of blood stage morphology and its responsiveness to currently used anti-malarial drugs. Thus, diagnosis of *P. knowlesi* infection in humans is usually unrecognized by routine laboratory tests based on blood film examination. The emergence of *P. knowlesi* malaria in humans signifies the importance of epidemiological studies of *P. knowlesi* among macaque populations, especially in Southeast Asia, and highlights the potential cross-species transfer of *Plasmodium* isolates. Meanwhile, *P. vivax* which causes morbidity in humans displays remarkable genetic relatedness to a number of malaria species infecting macaques; e.g., *P. knowlesi*, *P. cynomolgi*, *P. inui*, *P. fragile*, *P. simiovale*, *P. fieldi* and *P. coatneyi.* Hence, these macaque malarias are useful as out-group references for an estimation of the origin and evolutionary history of *P. vivax*. Sequence analysis of ~6 kb mitochondrial genomes of more than 100 worldwide isolates of *P. vivax* revealed that this human malaria parasite most likely arose from a host-switch from macaques to humans and that it underwent ancient population expansion around 200,000 to 300,000 years ago. Knowledge on the macroevolution of malaria is helpful in predicting the effectiveness of, and targeting antigen choice for, a vaccine derived from antigenic malarial proteins. If the origin of malaria is ancient the overall genetic diversity in

the extant malaria population is expected to be extensive making control by drugs and vaccine somewhat complicated and potentially ineffective. In contrast, if malaria originates very recently (within the past few thousands years), the extent of genetic and antigenic polymorphism in malaria proteins may be small. Consequently, the design of an effective vaccine should not be significantly compromised by the effect of polymorphism. This is because parasites with an ancient origin have plenty of time to accumulate nucleotide substitutions and amino acid replacements than those with a recent origin. Certainly the knowledge of malaria evolution is not merely of interest for evolutionary biology; its useful applications for rational malaria control range from policy for drug administration to slow down the evolutionary rate of malaria to the design of vaccine are tremendous. In the light of this, simultaneous studies of malaria in macaques or other non-human primates are of primary importance.

KEY WORDS: *Plasmodium knowlesi*, *Plasmodium vivax*, Evolutionary history, Nonhuman primate malaria, Host-switch

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A Comparison of Ketamine-propofol, Ketamine-isoflurane and Ketamine-xylazine for Anesthesia in Cynomolgus Monkeys (*Macaca fascicularis*)

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ABSTRACT.– Non-human primates are often used in preclinical research, and such studies frequently involve a variety of anesthetic conditions. However, few comparative studies have been carried out in cynomolgus monkeys. Therefore, it is important to evaluate the differences in cardiopulmonary measurements after introduction of volatile, intravenous, or intramuscular anesthesia.

Cynomolgus monkeys (females and males, 4-15 years old) reproduced in the Tsukuba Primate Research Center (Ibaraki, Japan) were used. In all monkeys, 0.05 mg/kg atropine and 10 mg/kg ketamine were given intramusculary as preanesthetic medication. Anesthesia was maintained with propofol (6-12 mg/kg/hour i.v.), isoflurane (0.5-1.5%), or xylazine (2.5 mg/kg i.m.). Blood pressure, heart rate, saturation pulse O_2, expiration of terminal CO_2 and body temperature were monitored.

Monkeys could be kept sedated over 2 hours by intravenous administration with 6-12-mg/kg/h propofol. In the period of anesthesia with propofol, mean heart rate was 134 (SD 13) bpm, mean systolic blood pressure was 81.3 (SD 11.4) mmHg. A significant fall in blood pressure occured during anesthesia with isoflurane and xylazine but not so marked with propofol. Furthermore, induction and recovery in anesthesia with ketamine-propofol were smooth and rapid even in long-term anesthesia. However, the analgesic effect with propofol might be not sufficient to allow surgical procedure to be carried out.

Propofol showed few depressant effects on cardiovascular function. Induction of anesthesia with propofol might be appropriate for non-invasive long-term research, for example magnetic resonance imaging studies.

KEY WORDS: Anesthesia, Propofol, Isoflurane, Ketamine

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Hybridization of Introduced Taiwanese Macaques with Native Japanese Macaques in Wakayama Prefecture, Japan

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ABSTRACT.– A group of Taiwanese macaques (*Macaca cyclopis*) escaped from a private zoo in the Ooike-yuuen area of Wakayama Prefecture, Japan in *c.a.* 1950. An adult male captured at Nakatsu village in 1998 was found genetically to be a hybrid between *M. cyclopis* and native Japanese macaques (*Macaca fuscata*). The existence of a free-ranging Taiwanese macaque population was subsequently confirmed near Ooike-yuuen area in 1999. The population has increased in size reaching estimated 239-250 individuals in 2002. Extensive hybridization in the population was speculated from observations of morphological variations in tail length and pelage color. After the prefectural government decided to eliminate the population, more than 240 individuals have been captured with the present population size estimated at 50-78, with an annual population growth rate of 14%.

We conducted a genetic assessment of the macaque population. The rate of hybridization and the breeding structure was examined using diagnostic molecular markers to blood proteins (ADA, DIA, TF), nuclear DNA (NRAMP1), Y-chromosomal DNA (TSPY) and mitochondrial DNA (mtDNA). From examination of 68 individuals captured during March-July 2003, evidence of extensive hybridization was found. Due to female philopatry, the use of sex-specific DNA markers (TSPY and mtDNA) was useful for discriminating birth place of individuals and immigrant male *M. fuscata*. Autosomal nuclear markers (blood proteins and NRAMP1) were used to assess the hybridization rate of each individual and breeding structure of the hybrid population. Approximately 85% of the monkeys were hybrids and 4% were immigrants of *M. fuscata*. The distribution of genotypes suggested that the 2 macaque species were intermixing extensively without reproductive constraints. This man made hybrid zone threatens to disturb the gene pool and biological features of native *M. fuscata*. The risk of expansion of exotic genes still remains serious in the surrounding area.

KEY WORDS: Hybridization, Japanese macaques, Taiwanese macaques, Wakayama Prefecture

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Review of the Literature on Primates of Bangladesh

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ABSTRACT.- We report here the result of a literature survey on Primates to reveal the potential change in primate populations in Bangladesh. Although recent information is available in the national and international scientific journals, old records could only be found in the district gazetteers since 1807 and local reports. Occurrence of rhesus macaque (*Macaca mulatta*) has been reported from greater Dinajpur and Rangpur districts since 1807; slow loris (*Nycticebus coucang*), capped langur (*Trachypithecus pileatus*) and hoolock gibbon (*Hylobates hoolock*) were reported from Chittagong and Sylhet by O'Malley and Risvi in 1808 and 1975 respectively, and long-tailed macaque (*Macaca fascicularis*) and common langur (*Semnopithecus entellus*) were reported from Jessore district since 1909. The remaining species of primates, pig-tailed macaque (*M. nemestrina*), Assamese macaque (*M. assamensis*), stump-tailed macaque (*M. arctoides*), phayre's leaf monkey (*Trachypithecus phayrei*) have been reported in recent years from 1978 to 1990. The observed trend was that first a general description on population status and habitat were published, and then, detailed and scientific studies such as on food and breeding activity in primates were published in more recent years by young and senior researchers.

It is noteworthy that the district gazetteers have called peoples' attention to the primate population decline from 1970 to 1992. However, little information is available on conservation issues of primates in Bangladesh. Although there are currently no nationwide censuses, it is highly probable that in recent years primate populations are sharply declining at an alarming rate. Slow loris, long-tailed and pig-tailed macaques, phayre's and common langurs and hoolock gibbon are designated as "critically endangered species", capped langur is "endangered", and rhesus macaque is "vulnerable". Scientists, researchers, national news media, etc. are shouting for conservation of primates. Not only the nationwide census of primate diversity and present status, but also the realization of preservation measures are both essential and urgently necessary. International economical and technical cooperation are essential.

KEY WORDS: District Gazetteer, Literature, Langurs, Loris, Gibbon

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Geoarchaeology of Thamlod Rockshelter, Mae Hong Son, Northern Thailand

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ABSTRACT.– We applied an integrated geoscientific approach to interpret the cultural chronology of the Thamlod prehistoric rock shelter site in Mae Hong Son area, northern Thailand. Generally, the study area is located in the Permain limestone with karstic topography. Geological, geochonological, and archaeological investigations constitute the essential methodology to examine land use pattern and natural resource exploitation in the past. Based on the results of on-site stratigraphy, geologic correlation, AMS and TL age dating, as well as physical and chemical analysis, four cultural chronological units (A, B, C and D) were identified. The oldest unit (Unit A) occurred in the early Late Pleistocene period (>32,000 yrs.), was created by natural depositional processes (non-cultural), and is characterized by lateritic soil in the lower part with a fining up sequence of gravel to sand layers in the upper part. Unit A indicates the past fluvial environment with paleo-chanel. Overlying Unit A is Unit B from the Late Pleistocene period (dated prior to ca. between 32,000 - 10,000 yrs. BP), which comprises layers of both natural and cultural depositional process. Cultural process is characterized by numerous remnants of stone tools (two of burial in the top layer), with animal, shell and fish remains. Animal remains were found in all excavated areas with most animal bones moderately well preserved and characterized by small to medium sized pieces of bone that were either burned or not burned. Random sampling of animal remains from areas of high deposition density of deposition was used to identify species, especially using the teeth. Tooth analysis grouped animals by size into 3 groups as follows. (i) Large animals, mostly cervid and bovid but surprisingly, bear was recognized in the deposit too. (ii) Medium sized animals which were mostly cervid, mountain goat, pig, rhizomys and small primates. (iii) Small animals, mostly lizard, fish, rat, reptile, crab, trionyx and birds. Shell remains were well preservedand characterized as burned or not. A preliminary analysis of a fresh water shell suggested *Nodularia scobinata (Carditidae)* as the species. The spatial distribution indicated that the site was used as a temporary camp and lithic workshop, a significant and good evidence of human occupation, and is an important clue to discriminate unit B from other units. The evidence of natural process was supported by weathered angular gravels to boulders of limestone, and deposited as lenses with an incline orientation. We hypothesize that the rock fall might have been caused by neotectonic or earthquake in the past - induced phenomena concurrent with those found in Krabi and Kanchanaburi in the same historical period. Unit C originated during the early to Middle Holocene period (dated prior to ca. between 9,980 - 2,900 yrs. BP) period. The deposition of unit C shows a time gap or

unconformity caused by flooding in the past, characterized by an increasing amount of organic matter and montmorillonitic clay. Archaeological evidence includes transported potsherds and beads. Therefore, the flooding process might be a major factor affecting the re-deposition of archaeological remains and mixing potsherd and beads together. The youngest of the four units, Unit D, occurred in the Late Holocene period (dated prior to ca. after 2,900 yrs. BP), was deposited as a topsoil layer, and contains archaeological remains such as potsherd, beads and iron tools that can be dated to late Holocene period.

KEY WORDS: Tham Lod, Rock shelter, Geoarchaeology, Mae Hong Son, Northern Thailand

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Population Dynamics of Japanese Macaques (*Macaca fuscata*) at Takasakiyama, Japan

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ABSTRACT.– Since 1953, a population of Japanese macaques (*Macaca fuscata*) has been fed artificially at Mt. Takasakiyama, which is surrounded by cultivated fields. The artificial feeding was done for visitors at an open zoo and to keep the animals away from cultivated fields. For more than 20 years they were given as much food as they could eat. Starting at around 200 individuals, the population dramatically increased, reaching over 2000 individuals by 1979, whilst the original troop had divided into three troops and peripheral animals began to invade fields to raid crops. Artificial feeding was gradually decreased from about 500 Kcal / day / head to less than 300. Body mass of adult females decreased, and the age of females at first birth increased. As a result, the birth and population growth rate both dropped, the former to about 40 %, which is a little higher than that of troops in natural conditions.

KEY WORDS: Population dynamics, Japanese macaques, Body mass, Birth rate

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Color Blind Monkeys Found in Indonesia

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ABSTRACT.- Old World primates have trichromatic vision because they have 3 types of cone photoreceptor maximally sensitive to long (L)-, medium (M)- and short (S)- wavelengths of light, respectively. Although a proportion of human males have X-linked color-vision abnormalities, no non-human Old World primates had been found to be color-vision defective. However, a molecular genetic-based analysis showed the existence of a dichromatic genotype of *Macaca fascicularis*. By using polymerase chain reaction (PCR) to specify genotype, we found male protanopes and female heterozygotes within some troops in Pangandaran National Park, Indonesia. The genome of male protanopes contains a single hybrid gene consisting of the 5' part of exons 1 to 4 of the L-photopigment gene and the 3' part of exons 5 and 6 of the M-photopigment gene (L4M5). The absorbance spectrum of the resultant L4M5 photopigment, characterized by photobleaching analysis, is very close to that of the M-photopigment. In the following analyses, we tested the retinal response of these monkeys by electroretinogram (ERG) flicker photometry. The results showed the low sensitivity to red light in protanopic males compared with normal monkeys. In female heterozygotes, sensitivity to red light was intermediate between the genetic protanopes and normal monkeys. We further assessed the behavioral phenotypes of monkeys carrying this hybrid gene by a pattern discrimination task with modified Ishihara pseudo-isochromatic plates. Normal monkeys and female heterozygotes could discriminate probe stimuli, but the protanopic males could not. The frequency of dichromats in these populations was 0.4%, which is lower than that found in humans. No dichromatic monkeys were found in the other macaque species tested. The results confirmed that protanopia monkeys have similar vision to human protanopia.

KEY WORDS: Color vision deficiency, Retina, Photopigment, *Macaca fascicularis*

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Preliminary Study on the Ecology of Leaf Monkeys (*Trachypithecus obscurus or T. phayrei*) in Popa Mountain Park, Myanmar

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ABSTRACT.– Since the leaf monkeys have been extensively hunted to obtain gallstones that is utilized as traditional medicine ("bezoar stone") as well as for food, it is rather difficult to observe wild troops in Myanmar. The leaf monkey troops of this study (*Trachypithecus obscurus or T. phayrei*) were found in the Popa mountain Park (sacred volcanic mountain, "reserve forest" established in 1902, "park" in 1982), an area of approximately 128.54 square km, located at 20° 53' N and 95° 15' E, in Kyauk Pan Daung Township, Mandalay Division. We report here our preliminary field survey in the Park and describe the fundamental ecological features as follows: 1) Three troops resided in the Nwa La Boh, Hmon Pya, and Say Hmon Valleys, respectively. 2) By both direct observation and fecal analysis, it was found that the leaf monkeys feed on more than 30 plant species. 3) Their habitats were the steep slopes of the valleys, and a mixture of microhabitats appears indispensable for their subsistence. These include big trees, various rocks, bushes or shrubs. Furthermore, we will report the population charactertistics, foraging patterns, and distribution of food plants.

KEY WORDS: The leaf monkey (*Trachypithecus*), Population richness, Habitat, Food Plants Species, Distribution in Myanmar

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Species Diversity and Distribution of Primates in Lao PDR

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ABSTRACT.– Non-human primates in Lao PDR have been recorded and studied since the 1940s, but most studies have been done in the 1990s, and mostly conducted in areas that are now protected. Seventeen non-human primate species have been recorded in Lao PDR in total, comprised of 6 species of Macaque Monkey (*Macaca sp.*), 3 species of Loris (*Nycticebus sp.*), 4 species of Langur (*Semnopithecus sp.* and *Pygathrix sp.*) and 4 species of Gibbon (*Hylobates sp.*). Most of these species were reported to be found mainly in the central and southern parts of the country, with the exception of gibbons that are distributed in all parts of the country. Despite the number of studies that have been conducted, there is still a clear lack of information about the number and variety of each species present, as well as their current geographical and ecological distribution within Lao PDR. Therefore, in July 2005 a primary survey was conducted by a team of Japanese and Lao researchers in 4 provinces of Lao PDR; namely Khammouane, Savannakhet, Saravan and Sekong. The survey was done by interview, observation and DNA sample collection. In interviews, a total of 12 species (2 species of *Nycticebus*, 4 species of *Macaca*, 3 species of *Semopithecus*, 1 species of *Pygathrix* and 2 species of *Hylobates)* were reported in the survey area. The presence of 7 of these species (4 species of *Macaca*, and 1 species for each of *Semopithecus*, *Pygathrix* and *Hylobates*) was confirmed by observation in the forest and in captivity. Samples (faeces, hair, etc) from 4 macaque species were collected and used for mitochondrial DNA based analysis. As for the present status of non-human primates in Lao PDR, principal current threats include wildlife trade (used for medicine and food and hunting for pet and local food.

KEY WORDS: Primates, Diversity, Distribution, Lao PDR

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Rhabdomyosarcoma in a Young Lion-tailed Macaque (*Macaca silenus*)

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ABSTRACT.– Rhabdomyosarcoma in non-human primates is very rare, but is of considerable interest from the viewpoint of comparative oncology. We report the histopathological and immunohistochemical features of a spontaneous rhabdomyosarcoma that occurred in a young male lion-tailed macaque (*Macaca silenus*).

A tumor in the left elbow of a four-year-old male lion-tailed macaque was removed surgically but had recurred one month later, leading to surgical amputation of the macaque's left arm. However, metastatic tumor developed in the left chest and the macaque died after a further seven months, X-ray examination revealed no evidence of metastasis to the bone. The gross morphology of the tumor was yellow-white in colour in cut surface with invasion into the surrounding muscle tissue. Histopathologically, neoplasm had proliferated in the muscle. A packed bundle of large polymorphic neoplastic cells, containing abundant eosinophilic cytoplasm and a round to ovoid, occasionally severe pleomorphic nucleus, were arranged in an interwoven pattern. Neoplastic cells had one or several very large prominent nucleoli in the nucleus. Multinuclear giant cells were scattered throughout the tissue. Immuno-histochemically, the neoplastic cells were intensively positive for desmin, myoglobin, creatinine phosphokinase (skeletal muscle type) and vimentin, but were negative for alpha-anti-chymotrypsin, lysozyme and the macrophage marker AM-3K.

Thus the rhabdomyosarcoma of this lion-tailed macaque corresponded to the pleomorphic type rhabdomyosarcoma of humans.

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KEY WORDS: Lion-tailed macaque, Juvenile, Rhabdomyosarcoma

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Fundamental Biological Features of a Troop of Long-tailed Macaques (*Macaca fascicularis aurea*) Inhabiting the Bayin Nyi Naung Mountain, Myanmar

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ABSTRACT.- **The long-tailed macaques in Myanmar are classified as a different subspecies (*Macaca fascicularis aurea*) to those found in other Indochinese countries (*M. fascicularis fascicularis*). However, their detailed biological features have not been reported yet. A troop of habituated long-tailed macaques that inhabit the Bayin Nyi Naung Mountain (BNNM), a steep limestone mountain in Kayin State (N 16° 58.2', E 97° 29.6') was studied. The troop is isolated from other populations by the Thanlwin River and its flood plain, and human settlements. We observed their morphology, population structure, reproductive seasonality, food plants, and social behaviors during January to July 2005. The tail length relative to head and body length was 93-104% and many individuals had infrazygomatic pattern of cheek hairs which are typical morphology of *aurea*, and no crest at the head was observed in many animals. The animals changed food seasonally, and forty species of food plants were identified. The births appeared to occur almost throughout a year. The relation between food availability and the timing of reproduction was ambiguous. More detailed study is needed to delineate the biological features of this subspecies and to consider preservation measures.**

KEY WORDS: Myanmar, *Macaca fascicularis aurea,* Biological features, Morphology, Social behaviours

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Status, Distribution and Habitat of Primates of Bangladesh

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ABSTRACT.– Ten species of primates are known to occur in Bangladesh. We report here the status, distribution and habitat condition of Bangladeshi primates based on both direct field observations in different districts of Bangladesh and on reviewing the published literature since 1970.

Slow loris (*Nycticebus coucang*) and phayre's leaf monkey (*Trachypithecus phayrei*) are rare, critically endangered nationally and unknown globally. Rhesus macaque (*Macaca mulatta*) is fairly common, vulnerable nationally and lower risk globally. Pig-tailed macaque (*M. nemestrina*) is few, critically endangered nationally and vulnerable globally. Stump-tailed (*M. arctoides*) and Assamese macaques (*M. assamensis*) are rare, data deficient nationally and vulnerable globally. Crab-eating or long-tailed macaque (*M. fascicularis*) is rare and localized, critically endangered nationally and lower risk globally. Capped langur (*T. pileatus*) is fairly common, endangered nationally and vulnerable globally. Common langur (*Semnopithecus entellus*) is endemic, limited in number, critically endangered nationally and lower risk globally. Hoolock gibbon (*Hylobates hoolock*) is few, critically endangered nationally and data deficient globally.

Slow loris, pig-tailed, stump-tailed, Assamese and crab-eating macaques, phayre's leaf monkey, capped langurs, and hoolock gibbon are widely distributed in the Chittagong Hill Tracts, Chittagong, Cox's Bazar and some of them in the greater Sylhet. Capped langur additionally is distributed in Madhupur National Park. Common langur is localized in Keshopur, Jessore district. Rhesus macaque is widely distributed in several districts of Bangladesh including Sundarbans mangrove forests and Dhaka City.

Most of the Bangladeshi primates occur in the tropical moist deciduous hilly forests or deciduous forests (*Sorea robusta*). However, common langur occurs in the human habitat of village woodlands and markets whilst rhesus macaque occurs in forests and town and city. Suitable primate habitats are decreasing everywhere due to the rapid increase of human population and activities including deforestation, exploitation and fragmentation.

Primate populations are also rapidly declining because of the habitat destruction, food shortage, trapping for zoos, circus, or street exhibition. Field study and research are essential for constructing effective preservation strategies and action plans to protect and manage the primate populations of Bangladesh. The poverty, illiteracy, and lack of awareness of the indigenous people underlies these threats to primates, and therefore, cooperation of international organizations, NGOs, etc. are essential in this regard.

KEY WORDS: Hanuman langur, *Sorea robusta*, Community

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A Preliminary Report of a Parasitological and Microbiological Based Survey of Free Ranging Japanese Macaques (*Macaca fuscata* (Blyth)) in the Boso Peninsula, Japan

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ABSTRACT.– **Because alien macaques (probably *Macaca mulatta*) occur in the southern part of the Boso Peninsula, Japan, there is a possibility that their parasites and/or other pathogens may infect Japanese macaques (*M. fuscata*). To test this hypothesis we have principally studied the parasites (protozoan, helminth and arthropod), but also other pathogens including *Rickettsia* spp. and the Borna virus, of 50 free ranging Japanese macaques in the peninsula. Two arthropod (*Pedicinus obtusus* and *P. eurygaster*) and three nematode (*Streptopharagus pigmentatus*, *Strongyloides fulleborni*, and *Trichuris trichiura*) parasite species were found on the Japanese macaque, in this survey. These arthropod and nematode parasites are not only common to Japanese macaques, but also to the other macaque species. To our knowledge, this is the first record of sites where *Pedicinus* spp. is seen in a host species. *Coccidia* sp. and *Babesia* sp. were not recorded. Results concerning other pathogens including *Rickettsia* sp., *Coxciella* sp. and the Borna virus from the same host samples are currently under investigation.**

KEY WORDS: *Macaca fuscata*, Parasites, Bacteria, Virus, Japan

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Monitoring Gonadal Functions in Non-human Primates by Means of Urinary and Fecal Steroids

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ABSTRACT.- Because of their close phylogenetic relationship to humans, higher primates have attracted considerable interest in a wide range of research areas. The results from studies of primate physiology have in particular made numerous significant contributions to our understanding of human evolution. However, with the serious decline of most non-human primates, to the critically endangered level in many cases, in an entirely different direction, such studies are also becoming increasingly important for conservation biology.

The application of noninvasive methods for measuring hormone levels in non-human primates has revolutionized primate studies. Aspects of the physiological status of non-human primates can be attained by quantitative measurement of estrone conjugates (E_1C), pregnanediol-3-glucronide (PdG), testosterone, cortisol, follicle stimulating hormone (FSH), and monkey chorionic gonadotropin (mCG) in their excreta. However, although these are non-invasive methods, their validity and widely applicability remain unknown. Analysis of our results derived from these methods suggested that 1) urinary and fecal steroid metabolites accurately reflected the same ovarian or testicular events as observed in plasma steroid profiles in captive Japanese macaques. Also, time lags associated with fecal measurements were one day after appearance in urine; 2) these noninvasive methods were applicable to wild and free-ranging macaques for determining reproductive status; 3) hormonal changes during menstrual cycles and pregnancy could be analyzed by measurement of FSH, CG and steroid metabolites in the excreta in captive great apes and macaques; and 4) hormone-behavior relationships of non-human primates in their natural habitats and social setting could be analyzed. In macaques, associations between maternal rejection and excreted estrogen, but not excreted progesterone were found. Moreover, in males significantly higher levels of fecal cortisol were observed in high-ranking males than lower ranking males. These results suggest that the non-invasive monitoring of excreted hormones provides a stress-free approach to the accurate evaluation of the reproductive status in non-human primates.

KEY WORDS: Estrone conjugate, Pregnanediol glucronide, EIA, Macaque, Ape

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Present Status of Primates, Especially Macaques in Vietnam

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ABSTRACT.– **In this report, we present the preliminary results of the cooperative macaque research project that has been implemented in Vietnam by the Saigon Zoo and Primate Research Institute, Kyoto University.**

Vietnam has a rich variety of animal species, especially primates and is regarded as one of the biological hotspots in SE Asia. At present, 21 primate species are recorded, including 2 lorises, 10 colobines, 5 macaques and 4 gibbons. The Vietnam government has paid special attention to the wildlife conservation and its effort has achieved some good results. However, deforestation, illegal hunting and trading of primates have rapidly reduced wild primate populations, especially macaque populations.

We have started to survey primates in the southern half of Vietnam. The distribution of the 5 macaque species, *viz,* long-tailed (*Macaca fascicularis*), rhesus (*M. mulatta*), pig-tailed (*M. nemestrina leonina*), stump-tailed (*M. arctoides*) and Assamese macaques (*M. assamensis*) have already been reported by some authorities, but these were not always in agreement with each other. For example, the most controversial issue is the distribution of rhesus and Assamese macaques in our survey localities. In the central highland area, it is highly probable that at least one of the two species is found, but local people interviewed referred to them simply as "yellow monkeys" which questions the accurate species identification. Which species are present thus remains to be determined. To this end we are now conducting questionnaire surveys in this area, and will soon be able to delineate the macaques' distribution in general.

Field studies to date revealed that forest habitat fragmentations have led macaque populations to become isolated with each other, whilst the pervasive hunting has made them very timid. We can observe them only in protected areas. Non-indigenous individuals (confiscated or pet) have been released into the wild troops and hybrids between some macaque species have consequently been encountered. As the conservation status of macaques are much lower than those of leaf-monkeys, snub-nosed monkeys, or gibbons, their conservation tends to be neglected. However, the local populations have their specific biological characters which are of importance in each species. Therefore, it is urgently needed for us to take appropriate measures to conserve macaque populations inhabiting the area outside protected areas.

KEY WORDS: Biological hotspot, Macaque population, Macaques' distribution.

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Primates of Phu Khieo Wildlife Sanctuary: A Long-Term Study of an Exceptionally Diverse Community

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ABSTRACT.– In 2000, a long-term research project, located in the Phu Khieo Wildlife Sanctuary, Northeastern Thailand, called the Primates of Phu Khieo was established by Dr. Andreas Koenig and Dr. Carola Borries of Stony Brook University, in cooperation with local Thai authorities and researchers. The project goal is to test current models regarding costs and benefits of group life, evolutionary ecology of female social relationships, and male and female reproductive strategies with sympatric leaf monkeys and macaques. The short-term aim is to investigate feeding ecology, feeding competition, and female social relationships of these monkeys, beginning with Phayre's leaf monkeys (*Trachypithecus phayrei*). A trail system of more than 70 km and covering an area of 1,300 ha. includes home ranges of many primate groups. Three leaf monkey groups have been habituated, and serve as the focus of ongoing data collection. Comparative data on diet, ranging, and social behavior is being collected, nutritional and genetic analyses are underway, and hormonal analysis is planned. The project's next phase will add comparative studies of Assamese macaques (*Macaca* assamensis) conducted by Dr. Julia Ostner and Dr. Oliver Scheulke of the Max-Planck Institute for Evolutionary Anthropology, Leipzig. Two Thai students have also benefited from the project with studies on leaf monkeys and gibbons. Additionally, the project has hosted three teaching workshops for Thai researchers, park officials, and students. The focus of these workshops has been the instruction of research techniques useful for the study of primates all over Thailand, with the goal of encouraging comparative research. The study was approved by the National Parks, Wildlife and Plant Conservation Department (DNP) with permission from the National Research Council of Thailand (NRCT). Research and workshops were supported through funds from the National Science Foundation (main sponsor), American Society of Primatologists, Cleveland Metroparks Zoo, Conservation International/ Primate Action Funds Program, LSB Leakey Foundation, National Geographic Society, and Wenner-Gren Foundation as well as MPI EVA Leipzig and Stony Brook University.

KEY WORDS: *Trachypithecus phayrei*, *Macaca*, Long-term research,
Primate diversity

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Gene Recombination of the α-Globin Gene Region in Long-tailed Macaques

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ABSTRACT.- **Humans generally have two α-globin gene loci on one chromosome. We found, however, that chimpanzees and orang-utans had triplicated α-globin gene haplotypes with a frequency of 0.80 and 0.20, respectively. Long-tailed macaques (*Macaca fascicularis*) from Malaysia, Indonesia and the Malay peninsula of Thailand also have triplicated the α-globin haplotype which occurs at high frequencies (0.25-0.50). On the other hand, triplicated haplotypes occurred at very low frequencies in the long-tailed macaques of the northern and eastern Thailand and the Philippines as well as in rhesus macaques (*M. mulatta*) from India and China, and in Japanese macaques (*M. fuscata*) with frequencies of 0-0.13.**

Retrosequence P117, which originated from mRNA encoding 117 amino acids, was inserted into the intergenic region of α-globin. This P117 retrosequence had a BamHI restriction site (GGATCC) not present in the intergenic region itself allowing a RFLP assay to calculate the frequency of insertion of P117 retrosequence. The P117 retrosequence was inserted at a high frequency in the long-tailed macaques of northern and eastern Thailand as opposed to a triplication of α-globin gene. The frequency was low in long-tailed macaques from the Malay peninsula of Thailand, Indonesia, Malaysia and the Philippines. It was inserted in rhesus macaques and Formosan macaques but not in Japanese macaques.

The small differences in the nucleotide sequences between the parental P117 gene and the retrosequence in the long tailed macaque and Formosan macaque indicate that the insertion occurred as recently as before speciation of the fascicularis group. Further, the P117 retrosequence was found in new world monkeys but with a 13 base pairs deletion. The insertion time was calculated at around 35 MYA or more before present.

It is concluded that the same retrosequence P117 was inserted independently to the fascicularis group of macaques and the new world monkeys and might be deleted in Japanese macaques. The frequency of triplication of α-globin gene and that of P117 retrosequence were different between the continental and Malay Peninsula area of Thailand.

KEY WORDS: α -Globin gene, Retro P117 gene, Retrosequence, Long-tailed macaque, New world monkey

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Lethal Outbreaks of Toxoplasmosis in Common Squirrel Monkeys

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ABSTRACT.– Squirrel monkeys (*Saimiri sciureus*, SM) are a small breed of primates. In Japan, 70 zoos have colonies, totaling about 1,000 monkeys. SMs are also becoming popular as pets. New world monkeys including SM are considered more susceptible to Toxoplasma than Old World monkeys. Here we report on two mass outbreaks of toxoplasmosis in which more than 70 SM died.

All dead SMs were dissected and 28 (6 from colony A and 22 from colony B) were examined histopathologically. Formalin-fixed liver and lung samples from 5 animals were used for nested PCR analysis using primer sets directed against the gene encoding the NTPase of *Toxoplasma gondii.*

In colony A, 59 SM died of toxoplasmosis in one year. In the worst outbreak, during early October when there were 83 SM in the facility, 50 of them died within 10 days, a fatality rate of 60%. Prior to this outbreak, two groups (of 61 and 19 monkeys respectively) were mixed. In colony B, 13 of 28 SM died in 16 months, including one outbreak in which 8 of a group of 16 died within a week (50% fatality rate). Before this outbreak, colony B was known to have suffered stress from cold climatic conditions. In SM in both colonies, clinical progress of the disease was acute and some animals were found dead without having shown disease signs. Disease outbreak was unrelated to the age or sex of affected animals. Of the dead animals, 16 out of 16 were seronegative to Toxoplasma using latex agglutination, but 10 of 15 were seropositive using a Western blotting test. Toxoplasmosis had not been detected in SM in either colony for more than five years. Macrofindings common to all dead SM were pulmonary edema, hepato- and splenomegaly, enlargement of mesenteric lymph nodes and pleural effusion, occasionally accompanied by ascites. Microscopically, in the 28 examined samples, the lungs were usually severely edematous, with multifocal hemorrhage and serofibrinous exudate in the alveolar spaces, and interstitial pneumonia. Focal accumulation of cellular debris in areas of inflammation was also seen, and macrophages and alveolar epithelial cells contained large numbers of tachyzoites. In the liver, multifocal necrosis, scattered small granulomas and occasional cyst formation in hepatocytes were seen, and tachyzoites were visible in Kupffer cells and hepatocytes. Numerous tachyzoites were observed in tissue stamps and smears of pleural effusions and tissue from the lung, liver, and multiple other organs. PCR: A single 275bp Toxoplasma-specific product amplified from lung and liver extracts by nested PCR was used to identify the genus of protozoa identified histologically.

The present study confirmed the high rate of susceptibility to and fatalities from toxoplasmosis in SM that have been previously reported. However, the present study concerns a much more severe outbreak and greater fatalities than in previous reports. The present study supports the notion that stress, such as cold climatic conditions or social instability caused by mixing two groups within a colony, could become a trigger for the onset of the disease, although this requires substantiation. For example, the source of contamination in SM in both establishments remains unclear, and so the possibility of horizontal infection cannot be ruled out from the conditions of occurrence.

KEY WORDS: Toxoplasmosis, Squirrel monkey, Outbreak, Spontaneous

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Secretion of Leptin throughout Pregnancy and the Early Postpartum Period in the Japanese Monkey (*Macaca fuscata*): Placenta as another Potential Source of Leptin

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ABSTRACT.– Leptin is one of the most important factors linking nutrition and reproduction. In the present study, plasma concentrations of leptin during pregnancy and early lactation in the Japanese monkey (*Macaca fuscata*) were determined. Plasma concentrations of gonadotropins, immunoreactive (ir-) inhibin, and steroid hormones were also measured. To evaluate the likely production site of leptin in placenta, term placenta was fixed, sectioned and stained using immunohistochemical techniques.

Plasma leptin significantly increased during the 2nd quarter of pregnancy and progressively increased thereafter throughout pregnancy. During the 4th quarter of pregnancy, leptin levels reached up to 89 and 64 times of those during pre-pregnancy and the 1st quarter of pregnancy periods, respectively. After parturition, the leptin level abruptly decreased. During the first 10 d of lactation, its average level decreased to that of the 2nd quarter of pregnancy. Plasma ir-inhibin and estradiol-17β were elevated throughout the pregnancy and decreased after parturition, both of them being positively correlated with leptin levels during the whole pregnancy and early lactation. Plasma progesterone significantly increased during the 1st quarter of pregnancy and were maintained at a higher level compared with pre-pregnancy but sharply decreased after parturition. Placental homogenates contain a large amount of leptin protein. Immunohistological examination revealed that leptin was localized in syncytiotrophoblast cells.

The results suggest that placenta could secrete a large amount of leptin and may be another source of leptin during pregnancy in *M. fuscata*. High correlations among leptin, ir-inhibin, and E_2 during these stages suggest that these hormones may have important roles on leptin secretion during pregnancy in *M. fuscata*. This study was partially supported by a Grant-in-Aid for Scientific Research (The 21st Century COE Program) from the Ministry of Education, Culture, Sport, Science and Technology of Japan

KEY WORDS: Leptin, Pregnancy, Placenta, Japanese monkey, Lactation

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Molecular Genetic Studies of Gibbon Phylogeny and Phylogeography

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ABSTRACT.– Noninvasive genotyping of hair samples in my laboratory with Rachel Simmons and Keri Monda promises to resolve long-standing questions surrounding gibbon phylogeny, species and subspecies identity, and phylogeography. Our analysis of the informative mitochondrial control region sequence supports the recognition of four living genera: *Hoolock* (Mootnick & Groves, 2005, formerly *Bunapithecus*), *Nomascus*, *Hylobates* and *Symphalangus*. Generally, recognized species and species-groups form well-defined haplotype clusters that permit the taxonomic identification of animals of unknown origin. Interspecific and intraspecific variation in *N. leucogenys*, *S. syndactylus* and *H. lar* will be described and used to assess existing subspecific taxonomy. In the case of *H. lar*, phylogenetic and haplotype network analyses did not find clear differences between the Thai subspecies *carpenteri* and *entelloides* but our sampling was compromised as the gibbons studied were of unknown geographic origin. Nevertheless, it is now clear that comparable sequence data from animals of known provenance will resolve many of the remaining issues regarding gibbon evolution and phylogeography, and help set conservation management priorities for the remaining wild and captive populations

KEY WORDS: Gibbon, Phylogeny, Phylogeography, Noninvasive genotyping

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Variability of Dental Dimension in Japanese Macaques (*Macaca fuscata*): Sex Difference, Geographical Variation, Insular Effect, and Subspecific Differences

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ABSTRACT.– **Dental morphology offers important characters for systematic classification of fossil specimenss. However, they are not always reliable characters since variants could arise from either different sexes of the same species or different closely related species. Variations in dental dimensions are also considered to be produced by 1) geographical variation (Bergman's rule), 2) insular effect, that is, insular populations tends to be smaller, and 3) subspecific difference. To evaluate the importance of each of these factors, knowledge of the variation of dental dimensions within a given species is indispensable, but has not been accumulated. Japanese macaques (*Macaca fuscata*) are an appropriate species to examine these factors. In the present study, we statistically examined dental dimensions of wild Japanese macaques (*M. fuscata fuscata*) collected from 13 localities (Shimokita, Shichigashuku, Kinkazan[1], Nikko, Bousou, Nagano, Hakusan, Kanagawa, Shizuoka, Shodoshima[1], Shimane, Takasakiyma, Koshima[1]) and *M. fuscata yakui* (Yakushima[1]). We measured mesio-distal length, bucco-lingual breadth, and others.**

1: insular population

KEY WORDS: Japanese macaques, *Macaca fuscata*, Geographic variation, Odontometry

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Gastric Carcinoma in a Japanese Macaque (*Macaca fuscata*): Is the Cardiac Region Predisposed Site of Gastric Carcinoma in Non-human Primates?

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ABSTRACT.– Gastric carcinoma is one of the most common malignant tumors in humans, especially in Japanese. The tumors frequently occur in the lesser curvature of the antropyloric region, and less frequently in the cardiac region in humans. There have been only a few reports of gastric carcinomas in non-human primates (NHPs) that share significant similarity in anatomy and physiology with those found in humans. We examined a case of gastric carcinoma in the cardiac region of a Japanese macaque (*Macaca fuscata*), and tried to compare its morphological features to those in humans.

An 18-year-old male *M. fuscata* displayed gradual weight loss with occasional vomiting about 1 year before death. The animal degenerated to frequent vomiting and severe anorexia 2 weeks before death. Grossly, there was a 2-cm tumor mass in the esophagus-cardia junction with a severe degree of cardial stenosis. The tumor bulged irregularly with a severe ulcer on the surface, and was ill-defined in the mucosa. Histologically, there was an infiltrative growth of poorly differentiated carcinoma in the mucosa of the cardiac region, invading deeply through the muscularis mucosae to the submucosa and muscular layers. The tumor cells showed marked cell atypia with various-sized and prominent nucleoli, and formed occasional glandular structures and squamous differentiation. There were frequent mitotic figures in the tumor. The tumor cells contained mucinous material in the cytoplasm as detected by the Alcian blue stain and periodic acid-Schiff (PAS) reaction. There was no neoplasm except the gastric tumor.

This tumor was diagnosed as gastric carcinoma originating from the cardiac region based on the site of occurrence and morphological features. We previously reported an advanced gastric carcinoma originating from the cardiac region, characterized by infiltrative growth with prominent glandular formation in a Brazza's guenon (*Cercopithecus neglectus*). Other 3 cases of gastric tumors were reported in the literature, all of which derived from the cardia. It is still uncertain why gastric carcinomas principally occur in the cardiac region in NHPs? This study was partially supported by a Grant-in-Aid for Scientific Research (The 21st COE Program) from the Ministry of Education, Culture, Sport, Science and Technology of Japan

KEY WORDS: Japanese macaque, Gastric carcinoma, Cardiac region

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Naturally-occurring Lesions in Japanese Macaques (*Macaca fuscata*)

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ABSTRACT.– In Japan an original species of macaque, Japanese macaque (*Macaca fuscata*; M.F.) are found endemic in Honshu, Sikoku and Kyushu islands, whilst the sub-species, *M. fuscata yakui* is found in Yaku-island to the far-south of Kyushu. Although the ecological, sociological and anthropological aspects of M.F. have been studied well, very little is known about their pathology including diseases and naturally-occurring lesions. We have examined more than 200 M.F. to ascertain their prevalent pathological features, information that will be of potential use for conservation and environment assessment. The M.F. specimens examined were derived from euthanised samples (population control) and naturally dead specimens in zoos and biomedical research breeding colonies.

In the respiratory system, anthracosis is commonly observed even in wild M.F. collected in rural mountainous areas. Grossly, the affected lungs were dark with black-coloured pulmonary lymph nodes. Histologically, various amounts of carbon pigment was accumulated around blood vessels and engulfed in the macrophages. In the pulmonary lymph nodes, large numbers of carbon-pigment laden macrophages accumulated in the lymph sinus. Electron microscopy confirmed numerous carbon particles in the cytoplasm of macrophages, whilst X-ray micro-analysis revealed prominent peaks of silicon and iron, thought to originate from soil dust. There were also frequent bronchopneumonia and labor pneumonia in wild and zoo-maintained specimens kept in open-air fields.

In the urinary system, wild M.F had frequent renal oxalosis, perhaps due to ingestion of oxalic acid -rich plants such as tree buds. Oxalosis was observed even in younger animals, with 18 percent of all monkeys examined being affected.

In the central nervous system, sporadic outbreaks of paralysis in the hind limb were observed in several macaques in a breeding colony from 1984. Histological examination revealed acute or chronic necrosis of white matter in the spinal cord, and necrotizing encephalitis in the medulla oblongata and cerebellum. Similar lesions were seen in some breeding colonies of M.F. in North America.

In the cardiovascular system, various degrees of myocardial fibrosis were observed andsecondary pericarditis due to pleuropneumonia. There were cases of arteriosclerosis of the aorta in wild-caught M.F. fed on leftover food from a restaurant. In the brain, there were frequent vascular mineralizations in the blood vessels in the globus pallidus, but no clinical signs were observed. There were several neoplastic lesions in wild and zoo-maintained M.F.; malignant lymphomas, trichoblastoma, odotogenic tumors and squamous cell carcinoma were seen.

Thus, pathological studies on wild and zoo-maintained M.F. will provide useful information for environmental assessment for humans, wildlife conservations by monitoring infectious diseases, nutritional disorders, inherited diseases and toxic diseases, and detection of zoonotic infectious diseases.

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KEY WORDS: Japanese macaques, *Macaca fuscata*, Pathology, Spontaneous lesions

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Preliminary Report on the Prevalence of the Parasitic Helminths Obtained from Pet Primates Transported into Japan

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ABSTRACT.– **Importation of pet primates presents a potential risk of importation of novel parasites (species or isolates) that may subsequently infect parasite-naïve wild primate populations. A survey of parasitic helminth infections was carried out on 96 individuals of pet animals belonging to 22 species in 5 families of the order Primate namely Lemuridae (*Lemur catta* and *L. fulvus*), Lorisidae (*Galago senegalensis*, *G. crassicaudatus*, *Nycticebus coucang*, *N. pygmaeus* and *Perodicticus potto*), Cebidae (*Aotus trivirgatus*, *Saimiri sciureus*, *Cebus apella* and *C. capucinus*), Callithichidae (*Cebuella pygmaea*, *Callithrix jacchus*, *C. penicillata*, *C. geoffroyi*, *Saguinus oedipus*, *S. midas* and *Leontidius chrysomelas*), and Cercopithecidae (*Macaca fuscata*, *Cercopithecus mona*, *Erythrocebus patas* and *Miopithecus talapoin*). In total, 13 nematodes (*Physaloptera*, *Rictularia*, *Dipetalonema*, *Gongylonema*, *Streptopharagus*, *Enterobius*, *Lemuricola*, *Crenosomatidae* gen., *Primasubulura*, *Globocephalus*, *Strongyloides*, *Molineuse* and *Trichuris*), 1 trematode (*Dicrocoelidae* gen.), 2 acanthoceplans (*Prosthenorchis* and *Nephridiacanthus*) and 2 pentastomid genera (*Armillifer* [?] and *Procephalidae* gen.) were recorded from 45 individuals from 13 species of pet primate. Except for *Enterobius* species, all helminths obtained are new records in Japan. Among these pet primates, all *S. sciureus* individuals examined were infested with *Enterobius* sp. and/or *Strongyloides* sp. The talapoin monkey (*M. talapoin*) was the pet primate with the highest prevalence of helminth parasites, being parasitized by 12 helminth groups. Since primate is one of the most favorite pet animals in Japan, a concern should be raised over this potential health risk to the wild primate populations.**

KEY WORDS: Primates, Helminths, Pets, Japan

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Nonhuman Primates (NHP) as Models of Human Diseases

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ABSTRACT.– **Among 200 species of NHPs, several monkeys and great apes are used for biomedical research. They are chimpanzees, and rhesus, cynomolgus and Japanese macaques which belong to the Old World monkeys, and the New World monkeys such as squirrel monkeys and common marmosets.**

NHPs have advantages over other biomedical research mammals such as rodents in the ability to more reliably extrapolate their data directly to humans, because they have a closer genetic, morphological, and physiological relationship to humans than other experimental animals. Thus, infectious, senile or neurological disease models have been developed using NHPs. They are also used for translational studies of new medical technologies.

However, NHP also have some weak points, such as: they are expensive, hard to handle, the absence of genetic and biological controls, the high risk of zoonoses, need for compliance for CITES, legal quarantine before use etc. Despite these, and other disadvantages, NHP are still necessary for biomedical researches.

The key point of any NHP animal model is as follows. 1) The same animal model cannot be obtained in rodents. That is if the model is obtainable in rodents, then there is no need to develop a NHP animal model because it is easier to get results from rodents than from NHPs. 2) The NHP model covers data which cannot be obtained from patients. That is if the data can be obtained from patients directly, it is easier to get results from patients than from NHPs. Only the NHP models which have the above two characters may be a breakthrough of biomedical sciences. In this symposium, I would like to introduce several NHP animal models including AIDS, EHEC, AZD, AMD, Gene therapy models in which we are conducting our projects.

KEY WORDS: Non-human primates disease models, AIDS, AZD, Brain infarct model.

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