Published in final edited form as:

Am J Primatol. 2014 November ; 76(11): 1094–1104. doi:10.1002/ajp.22296.

Diversity and Molecular Phylogeny of Mitochondrial DNA of Rhesus Macaques (*Macaca mulatta*) in Bangladesh

M. KAMRUL HASAN^{1,*}, M. MOSTAFA FEEROZ², LISA JONES-ENGEL³, GREGORY A. ENGEL^{3,4}, SREE KANTHASWAMY¹, and DAVID GLENN SMITH¹

¹Molecular Anthropology Laboratory, Department of Anthropology, University of California, Davis (UC Davis), California

²Department of Zoology, Jahangirnagar University, Savar, Dhaka, Bangladesh

³University of Washington, National Primate Research Center, Seattle, Washington

⁴Swedish Medical Center, Seattle, Washington

Abstract

While studies of rhesus macaques (*Macaca mulatta*) in the eastern (e.g., China) and western (e.g., India) parts of their geographic range have revealed major genetic differences that warrant the recognition of two different subspecies, little is known about genetic characteristics of rhesus macaques in the transitional zone extending from eastern India and Bangladesh through the northern part of Indo-China, the probable original homeland of the species. We analyzed genetic variation of 762 base pairs of mitochondrial DNA from 86 fecal swab samples and 19 blood samples from 25 local populations of rhesus macaque in Bangladesh collected from January 2010 to August 2012. These sequences were compared with those of rhesus macaques from India, China, and Myanmar. Forty-six haplotypes defined by 200 (26%) polymorphic nucleotide sites were detected. Estimates of gene diversity, expected heterozygosity, and nucleotide diversity for the total population were 0.9599 ± 0.0097 , 0.0193 ± 0.0582 , and 0.0196 ± 0.0098 , respectively. A mismatch distribution of paired nucleotide differences yielded a statistically significantly negative value of Tajima's *D*, reflecting a population that rapidly expanded after the terminal Pleistocene. Most haplotypes throughout regions of Bangladesh, including an isolated region in the southwestern area (Sundarbans), clustered with haplotypes assigned to the minor haplogroup Ind-2 from India reflecting an east to west dispersal of rhesus macaques to India. Haplotypes from the southeast region of Bangladesh formed a cluster with those from Myanmar, and represent the oldest rhesus macaque haplotypes of Bangladesh. These results are consistent with the hypothesis that rhesus macaques first entered Bangladesh from the southeast, probably from Indo-China, then dispersed westward throughout eastern and central India.

Keywords

rhesus macaque; *Macaca mulatta*; mtDNA; haplotypes; HVR 1; Bangladesh

*Correspondence to: M. Kamrul Hasan, Molecular Anthropology Laboratory, Department of Anthropology, University of California, Davis (UC Davis), CA 95616. mkhasan@ucdavis.edu.

INTRODUCTION

The genus *Macaca* is believed to have originated in northern Africa as early as 7 million years ago (Mya) [Eudey, 1980] and migrated through the Middle East and northern India approximately 3 Mya. Macaques had passed through most of China and reached the Indonesian archipelago as early as 2.5 Mya where the common ancestor of the *fascicularis* species group originated [Abegg & Thierry, 2002; Delson, 1980; Fooden, 1976]. It has been hypothesized that rhesus macaques diverged from a fascicularis-like ancestor that reached the mainland of Indo-China from Indonesia by approximately 1 Mya [Delson, 1980; Fooden, 2006; Stevison & Kohn, 2009; Tosi et al., 2003]. They subsequently dispersed throughout most of Asia but the origin, route, and timing of this dispersal is not well understood.

The marked genetic homogeneity of Indian rhesus macaques [Smith & McDonough, 2005] may result from their location at the terminus of a serial founder effect from the westward dispersal of the species, as reported for modern human populations expanding eastward from Africa [Ramachandran et al., 2005]. Although a genetic study was conducted on a small population of free ranging Nepali rhesus macaques [Kyes et al., 2006], studies of genetic variation of rhesus macaques has primarily focused on animals in captive breeding facilities, limiting such studies to rhesus macaques originating in India and China [Smith & McDonough, 2005]. Unfortunately, virtually no molecular research on rhesus macaques of Bangladesh has been reported. A preliminary survey of the mtDNA diversity in 39 individuals from five different localities of Bangladesh [Feeroz et al., 2008] detected a total of only seven haplotypes. Recently, we conducted a study of rhesus macaques and associated foamy virus in Bangladesh, and used microsatellites to characterize population structure in macaques from six urban populations [Engel et al., 2013; Feeroz et al., 2013]. This study found the monkeys in a central population (Madhupur) to reflect admixture with the Sundarbans population and suggested that contemporary human agency such as monkey performers and monkey pet owners are playing a role in the genetic and viral composition of some of these populations.

Bangladesh is located at the transitional zone between the Indo-Himalayas and Indo-China sub- regions [Stanford, 1991]. Macaque migrations from west to east and vice versa were physically impossible without traversing Bangladesh. However, Bangladesh is a small and densely populated country that is crisscrossed by hundreds of rivers that restrict the distribution of primates. Its total area of 147,570 km² is home to more than 160 million people (1,084 people/km²). Many primate habitats have been destroyed by increasing human population pressure, industrialization, and rapid urbanization [Feeroz et al., 2011], and many rhesus macaque populations have been confined inside human settlements [Hasan et al., 2013].

Bangladesh currently supports five species of macaques: rhesus macaques (*Macaca mulatta*), pig-tailed macaques (*Macaca leonina*), cynomolgous macaques (*Macaca fascicularis*), Assamese macaques (*Macaca assamensis*), and stump-tailed macaques (*Macaca arctoides*) [Feeroz, 2001; Feeroz et al., 2011; Hasan, 2003; Khan, 1982]. The latter four macaque species are distributed only in the northeastern and southeastern hill areas of

the country and their population density is very low [Islam et al., 2000]. Rhesus macaques are found throughout the country except for the northwestern part where no primate species lives [Hasan et al., 2013; Khan, 1982]. This species is found in all types of natural forest, tea gardens, planted forests, and human settlement areas and is the only primate species found in the Sundarbans mangrove forest in the southwest of the country [Hasan et al., 2013]. Rhesus macaque populations in natural habitats of the country can be divided into the three major sub-populations defined by geographic and anthropogenic barriers shown in Figure 1: (i) central, (ii) eastern, and (iii) southwestern. The eastern sub-population is connected by forest that traverses the state of Tripura in Northeast India (see Map), which borders Bangladesh to the north, south, and west, and the Indian state of Mizoram to the east, dividing eastern Bangladesh into a northeast and southeast sector.

Detailed molecular research on rhesus macaque populations of Bangladesh could address some questions about the origin and distribution of rhesus macaques in their geographic range. The present study focuses exclusively on genetic variation in the mitochondrial DNA of rhesus macaque populations within Bangladesh and comparison of this variation with other populations of the species' geographic range. We characterized the (a) population structure and genetic diversity of rhesus macaques of Bangladesh; (b) geographical distribution of genetic variation; (c) genetic diversity within and between the local populations of rhesus macaques; and (d) phylogenetic relationship of Bangladeshi rhesus macaques to the other rhesus macaque populations in their geographic range.

We hypothesize that (i) the rhesus macaque population of Bangladesh comprises three sub-populations isolated from one another by river barriers and (ii) rhesus macaques expanded to Bangladesh from the east, expanded rapidly during Holocene times and dispersed westward along the Indo-Gangetic Plain and Indus River valley to their present geographic range through what are now India, Pakistan, and Afghanistan. We predict that (i) rhesus macaque sub-populations in Bangladesh will be genetically distinct from each other and that genetic distances among them will not be highly correlated with corresponding geographic distances due to river barriers to gene flow; (ii) Tajima's test of selective neutrality will exhibit a negative value of D characteristic of a rapidly expanding Bangladeshi rhesus population; and (iii) the southeastern portion of the eastern sub-population will exhibit genetic similarity to Myanmar rhesus macaque populations and contain the greatest genetic diversity while the central and western population of Bangladeshi rhesus macaques will be more genetically similar to Indian rhesus macaques.

METHODS

Sample Collection

Swabs from the surface of 86 fecal samples and 19 blood samples were collected from 25 local free ranging rhesus macaque populations belonging to the three sub-populations of the country from January 2010 to August 2012 (Table I). Each of the three sub-populations is geographically isolated from each other by physical barriers to natural dispersion (e.g., major river systems) and/or subsequent anthropogenic effects (e.g., habitat destruction resulting in a lack of natural corridors for movement). Fecal swabs were fixed in lysis buffer (1 M Tris-HCl, 0.5 M EDTA, 5 M NaCl and SDS). The animals in some populations were

trapped for blood samples and released to the same population. Sampling was conducted under the University of Washington Institutional Animal Care and Use Committee and Bangladesh Ministry of Environment and Forest Permit. Detailed trapping and sampling techniques were previously reported in Jones-Engel et al. [2006] and Feeroz et al. [2013]. Biological samples were transported to the United States under the permission of Convention on International Trade in Endangered Species.

Laboratory Analyses

The Promega PCR Clean-up System (Promega, Madison, WI, USA) was used to extract DNA from the fecal swabs using the manufacturer's protocol. The QIAGEN (QI Aamp DNA Blood Mini Kit, Qiagen, MD, USA) kit was used for blood DNA extraction. Extracted DNAs were quantified using the Qubit dsDNA HS Assay kit and diluted to 10 ng/μl for PCR amplification. We analyzed 762 base pairs (bp) of the non-coding D-loop of mtDNA (nps 15,777–16,539), including hypervariable region I (HVR I) and a portion of cytochrome *b* gene. The mtDNA sequences were amplified using primers 15167F (5'–3') ATGCAAGGCGCCACGATTT and 16050R (5'–3') CCGAGCGAATGCCACC. This primer pair was designed to replace a pair of previously used primers that readily amplified a pseudogene (numtDNA) [Smith & McDonough, 2005]. This pseudogene [Bensasson et al., 2001; Collura & Stewart, 1995] was easily identifiable by its extreme divergence from all rhesus sequences amplified with the redesigned pair of primers, an abundance of transversions and indels, a paucity of polymorphic, but consistently heteroplasmic, sites and the failure of its HVS I to readily align with the reference *Macaca sylvanus* sequence. The redesigned primers yielded sequences with specific mutations that provide the haplogroup structure characteristic of true mtDNA sequences, with the majority of the variation in the HVR I region [Smith & McDonough, 2005]. This conspicuous difference between the pseudogene and the mtDNA fragments amplified provided assurance that pseudogene sequences were not included in the sequences analyzed in this study.

For amplification, 2 μl of DNA template was used in a 25 μl of PCR reaction (nuclease free water, 10 μM of dNTP, 10 × PCR buffer, 50 μM of MgCl₂, 10 μM of forward and reverse primers and Platinum *Taq* DNA polymerase). Amplification was carried out under the following thermocycler conditions: 95°C for 3 min, 36 cycles of 95°C for 20 sec denaturing, 55°C for 10 sec annealing, and 72°C for a 40 sec extension). Successful amplification was confirmed by the identification of a fragment of the appropriate size after 6% polyacrylamide Minigel electrophoresis. PCR products were cleaned-up using 2 μl of ExoSAP-IT to 5 μl of PCR product at 37°C for 15 min followed by 15 min at 80°C.

Sequencing was performed on an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA) using BigDye Terminator v3.1 (Applied Biosystems) reaction mix, 5× BigDye sequencing buffer, 1 μM of forward and reverse primers, Exo-SAP-IT PCR product and nuclease free water. The thermocycler conditions were 96°C for 1 min denaturing, 25 cycles of 96°C for 10 sec, 50°C for 5 sec, and 60°C for 4 min. BigDyeX Terminator solution and SAM solution were added to the PCR product and rigorously vortexed for 30 min for the purification of the PCR products. The electropherograms were aligned with Sequencher v5.0 (Gene Codes Corp., Ann Arbor, MI).

Data Analysis

The 105 Bangladeshi rhesus macaque sequences were compared with 109 Indian, 21 Nepali, 13 Myanmar, and 88 Chinese rhesus macaque sequences that have already been described [Kyes et al., 2006; Smith & McDonough, 2005] (Fig. 2). Phylogenetic analyses were carried out in MEGA v5.2 using the maximum composite likelihood distance model, and a neighbor-joining (NJ) tree was constructed from a bootstrap analysis with 1,000 replicates. Other distance models employed in MEGA v5.2 generated trees with identical topology and near-identical bootstrap values. The ARLEQUIN software package (version 3.5.1.2) was used to estimate haplotype frequencies and molecular diversity indices and construct a mismatch distribution. Network 4.6.1.1 was used to construct a median-joining (MJ) haplotype network rooted with the *M. sylvanus* reference sequence cited above.

This research adhered to the American Society of Primatologists principles for the ethical treatment of non-human primates. The captured animals employed have been managed in compliance with Institutional Animal Care and Use Committee (IACUC) regulations.

RESULTS

Genetic Variation Within Bangladesh

Overall genetic variation—A total of 46 mtDNA haplotypes was detected in 25 local rhesus populations in Bangladesh (Table I). GenBank accession numbers for these sequences are KJ767675–KJ767715. A total of 200 (26%) of the 762 nucleotide sites studied were polymorphic and no indels (insertion/deletion) were observed. Most of the substitutions (78.2%) were transitions resulting in a transition: transversion ratio of approximately 3.6:1. A gene diversity of 0.9599 ± 0.0097 and expected heterozygosity of 0.0193 ± 0.0582 were estimated for the total population. The mean number of pair wise differences and average nucleotide diversity among the 762 bp were 14.0766 ± 6.3639 and 0.0196 ± 0.0098 , respectively. Nucleotide composition was C (28.21%), T (27.12%), A (32.41%), and G (12.27%). The negative value of Tajima's *D* ($D = -2.1104$) for haplotypes of rhesus macaques of Bangladesh was statistically significant at the 0.01 level of probability with an excess of haplotypes of low frequency, reflecting a geologically recent history of rapid population expansion.

Distribution of Haplotypes

MtDNA haplotypes of the three rhesus sub-populations of Bangladesh formed the three distinct haplogroups in the haplotype network shown in Figure 3.

Haplogroup 1—This haplogroup includes haplotypes from all three sub-populations. These include seven haplo-types from the eastern sub-population, five haplo-types from central sub-population and two haplotypes from the southwest sub-population. This is the oldest of the three haplogroups; haplotypes BR43, BR44, BR45, and BR46 are the oldest haplotypes when the haplotype network is rooted with the *M. sylvanus* reference sequence (NC002764) as shown in Figure 3.

Haplogroup 2—This haplogroup predominantly comprises haplotypes from the southwest sub-population but also included haplotypes from each of the two other sub-populations. Most of the haplotypes of the southwest sub-population diverged from a single haplotype of the central sub-population (BR23) in the haplotype network (Fig. 3) that is also the root of some haplotypes from the eastern sub-population (BR14, BR31). The Sundarbans sub-population (Southwest), which is 256 km from the central sub-population and crisscrossed by many large rivers, is isolated by many anthropogenic factors, but three of its haplotypes (BR24, BR25, and BR32) are derived from a single central haplotype, BR23 (Fig. 3). Haplotype BR1 of this haplogroup was shared by all three sub-populations including the central sub-population, which is about 50 km away from the other sub-populations, and located on both sides of a large river (the Meghna).

Haplogroup 3—This haplogroup primarily comprises haplotypes from the eastern sub-population (11 haplotypes) but also included two haplotypes (BR6 and BR15) from the southwest sub-population (Sundarbans) and four haplotypes from central sub-population as illustrated by the haplotype network (Fig. 3). Three haplotypes (BR34, BR35, and BR37) of the central sub-population and two haplotypes (BR11 and BR33) of the northeast sub-population derive from a single haplotype (BR15) of the Sundarbans (southwestern) sub-population, though these sites are 200–260 km apart and isolated from each other by many rivers and other barriers.

The phylogenetic (NJ) tree of the haplotypes from these three sub-populations formed five distinct sub-clusters (Fig. 4). Consistent with the haplotype network (Fig. 3), the haplotypes from the eastern (BR45 and BR46) sub-population formed the root of this tree. All the remaining haplotypes except those from the southeast and two from the northeast (BR43 and BR45) formed a single sub-cluster with low bootstrap value. Most of the haplotypes of the studied populations exhibited locality-specific distributions. Except for BR1, BR17, and BR31, each of the haplotypes is unique to a local population of rhesus macaques in Bangladesh. BR1 was shared by four different local populations (Kolargaon, Kartikpur, Nandanshar, and Naria) of the southwest sub-population, one local population (Chandpur) of the eastern sub-population, and one local population (Narayanganj) of the central sub-population of the country. BR17 was shared by two local populations (Khadimnagar and Syed Jahan shrine) and BR31 was shared by three local populations (Kalenga, Fenchuganj, and Malnichara) of the eastern sub-population. Approximately 64% of the 25 local populations studied exhibited more than a single haplotype.

Relationship Between Geographic Distance and Genetic Distance

The sampling locations of rhesus macaques in Bangladesh are separated from each other by geographic distances by between 3 and 393 km. In some locations where local populations of rhesus macaques were separated from each other by a short geographic distance, human settlements, and/or river systems of the country precluded gene flow between them. The correlation between the geographic and genetic distances among these local populations was positive and statistically significant ($r = 0.1726$, $P < 0.001$) as shown in Figure 5. However, when the southeastern sub-population is omitted from the analysis, the correlation between genetic and geographic distance is not statistically significant, suggesting that the

southeastern sector of the eastern sub-population is the population least effectively isolated by geographic barriers.

Relationship With Rhesus Populations Outside Bangladesh

When the haplotypes of Bangladeshi rhesus macaques were compared with the available sequences for rhesus macaques in other populations [Kyes et al., 2006; Smith & McDonough, 2005], three haplotypes (BR46, BR43, and BR45 from east) formed a separate sub-cluster representing the root of the NJ tree in Figure 6 with a 95% bootstrap value. Most of the remaining Bangladeshi haplotypes formed a separate sub-cluster with haplotypes of the Ind-2 haplogroup of India defined by Smith and McDonough [2005]. Three haplotypes (BR40, BR41, and BR42) from the southeastern part of Bangladesh clustered with all the Myanmar haplotypes. One haplotype (BR44) from the southeast formed the root of all Chinese samples (though with a very low bootstrap value). All the haplotypes from haplogroup Ind-1 from India [Smith & McDonough, 2005] formed a separate sub-cluster that included none of the Bangladeshi haplotypes. All the rhesus populations of Bangladesh (except one northeast local population and all southeastern populations) formed a single monophyletic sub-cluster, suggesting the monophyletic origin of most rhesus macaques in Bangladesh.

DISCUSSION

Haplotype diversity among the rhesus macaques of Bangladesh exhibited a locality-specific distribution. All but three of the haplotypes (BR1, BR17, and BR31), each shared by the adjoining local populations, except Narayanganj, represented a specific local population. Historically, no forest existed in Narayanganj and its vicinity, and the area has been used as a river port and business hub for at least 1,000 years [Islam et al., 2003]. Among the four haplotypes from Narayanganj, two haplotypes (BR1 and BR5) resembled those from the sub-populations of other riverine areas (Chandpur, Kolargaon, Nandanshar, Naria, and Kartikpur), but two others (BR38, BR39) resembled those from the eastern sub-population. Human-aided dispersal of monkeys to Narayanganj may have been facilitated by the area's ancient status as a river port and business hub. Significantly, none of the many rhesus macaque local populations between Narayanganj and the eastern region of the country resembled the Narayanganj population. This result is consistent with our recent report [Feeroz et al., 2013] in which we discuss Narayanganj as Bangladesh's "melting pot" for released and/or escaped pet macaques.

Sundarbans is the largest continuous mangrove forest in the world, covering an area of 6,017 km² and crisscrossed by four major rivers and their tributaries [Barlow et al., 2011; Khan, 2011]. In spite of geographic and anthropogenic barriers, the rhesus macaque population from Sundarbans (Karamjal) resembled those of central (Madhupur) and northeast (Satchari, Kalenga, Fenchuganj, and Malnichara) Bangladesh. These three sites are geographically isolated from each other with several rhesus macaque local populations intervening among them whose haplotypes lack resemblance to those from Sundarbans. Madhupur forest was located on the west side of the main channel of Brahmaputra. The main channel of the Brahmaputra was shifted southward, opening the present main channel (Jamuna in

Bangladesh), due to the tectonic uplift of the Madhupur Tract during the 1,762 earthquake episode, placing Madhupur forest in between old and present channels of Brahmaputra [Bhuiyan et al., 2010; Sussan et al., 1904]. The genetic resemblance of rhesus macaques from Madhupur region to Sundarbans in the southwest is reasonable because the Brahmaputra was not a barrier between these two regions before 1,762 earthquake. The intervening local populations between Sundarbans and Madhupur may have expanded following a different dispersal route. Large areas of Bangladesh have been deforested over the last two decades. The moist deciduous forests of Bhawal and Madhupur once covered much of the areas between the Padma, Jamuna, and Meghna rivers [Feeroz et al., 2013]. This extensive forest cover throughout the central Bangladesh would have allowed movement of rhesus macaques.

As in other Asian countries, non-human primate (NHP) pets are not uncommon in Bangladesh. Owners of NHP pets face a severe problem when confronted with a monkey acquired as an infant that has grown into an aggressive adult [Feeroz et al., 2013] and, consequently, usually release their adult pets in the nearest NHP population. In addition to NHP pet ownership, performing monkey ownership is a centuries old tradition in Bangladesh. Owners of performing monkeys usually capture monkeys from the forests and urban monkey habitats and train them for performance, and monkey trading is common among the monkey performers' community [Akhtar, 2014; Feeroz et al., 2013]. Release of untrainable monkeys to the nearest monkey population by this community is also common [Akhtar, 2014]. That Sundarbans (Karamjal) is the major source of the performing monkeys [Engel et al., 2013; Feeroz et al., 2013] is consistent with our findings. However, Karamjal is situated at the northern periphery of Sundarbans and its rhesus macaques may not be representative of those of the entire Sundarbans. Detailed sampling throughout the Sundarbans could clarify the rhesus macaque distribution in this remotest forest as well as in its geographic range.

The haplotypes from Chandpur, another old river port, and Shariatpur (Nandanshar, Kolargaon, Kartikpur, and Naria), geographically isolated from each other by the huge river Meghna (about 5.5 km width), resembled each other and include a common haplotype (BR1). The extension of the Meghna into India (the Brahmaputra river) has been argued to provide a barrier to gene flow separating the two major subspecies of rhesus [Melnick et al., 1993] and Assamese [Fooden, 1988] macaques. Monkeys might cross this river as stow-aways on water vehicles, by river rafting or with the aid of humans. If, as our analysis suggests, the rhesus macaques in these two areas share a recent common ancestry, the Meghna has not provided a barrier to gene flow, perhaps due to meandering of its tributaries and/or their circumnavigation downstream toward the Delta where the tributaries narrow.

All the southeast rhesus macaque populations of Bangladesh and a single local population of the northeast (Satchari) resembled each other, forming a separate sub-cluster. Though the northeast (Satchari) and the southeastern populations are about 250 km from each other, these regions are connected to each other through the hill forest of Tripura of eastern India (Fig. 1). Haplotypes from the eastern region of Bangladesh are the oldest haplotypes and are genetically distant from the other haplotypes of the country. The southeast region of Bangladesh is close to and continuous with the Aracan hill range of Myanmar, and the

sequences of rhesus macaques in the two areas form a cluster, apparently reflecting their recent common ancestry. As the southeastern sector of the eastern sub- population is the oldest and rhesus macaques in Bangladesh rapidly expanding in the recent past, rhesus macaques probably first expanded northward and westward through the country from southeastern Bangladesh.

According to Smith and McDonough [2005], rhesus macaques throughout their geographic range formed at least four distinct clusters (i.e., haplogroups) of mtDNA: Ind-1, Myanmar-Ind-2 in India and ChiE and ChiW in China/Vietnam. They argued that the Ind-2 haplogroup, comprising approximately 5% of the Indian haplotypes, derived from a recent westward dispersal of rhesus macaques from Myanmar. When we compared our sequences with those of Smith and McDonough [2005], we found five distinct clusters: (a) a separate Bangladesh cluster; (b) a Myanmar–Bangladesh cluster; (c) a Bangladesh–India-2 cluster; (d) an India-1 cluster; and (e) a China–Vietnam cluster. Ind-2 haplotypes from widely geographically dispersed locations in India (three from New Delhi, one from Kashmir, and another from Uttar Pradesh) clustered with some Bangladeshi rhesus macaque sequences (cluster c, above). These three Ind-2 sampling sites are very far from Bangladesh sampling sites, and none of the Ind-1 haplotypes resembles any of the Bangladeshi or Ind-1 rhesus haplotypes. Because the samples used by Smith and McDonough [2005] were collected from captive breeding centers, not directly from the wild, the validity of their alleged origin locality is problematical. The captive breeding centers collected Indian rhesus macaques from dealers before 1978, when regional origin was not regarded as important and may have been misrepresented. If valid, the wide geographic distribution of both Ind-1 and Ind-2 in India might reflect two very early westward dispersals of rhesus macaques at two different times and/or by two different routes. Samples of rhesus macaques from wild populations of Assam, Meghalaya, Tripura, and other regions adjoining Bangladesh may clarify this hypothesis.

Macaca mulatta and *M. fascicularis* probably diverged during middle Pleistocene times from a common fascicularis-like ancestor [Deinard & Smith, 2001]. Smith and McDonough [2005] argued that the current geographic distribution of rhesus macaques in India resulted from a westward dispersal, or redispersal, that postdates the species' dispersal through mainland Southeast Asia and China. Melnick and Kidd [1985] suggested that genetic similarity between Chinese rhesus macaques and cynomolgus macaques in Thailand results from the divergence of rhesus from cynomolgus macaques in Thailand during a glacial maximum, followed by the dispersal of cynomolgus macaques to the south. We found that the oldest known rhesus macaque haplotypes of the species geographic range are those from Bangladesh, which may support the argument that rhesus macaque originated in rainforests of Chittagong Hill Tracts in southeast Bangladesh that include Aracan Hill Range of Myanmar and dispersed eastward towards China–Vietnam and westward toward western India as hypothesized by Smith and McDonough [2005]. If so, a refuge may have existed in Chittagong Hill Tracts-Aracan Hill Range during either the last or penultimate glacial maximum. The latter is more consistent with the 162,000 years BP divergence date between Indian and Chinese rhesus macaques estimated by Hernandez et al. [2007] based on 1,467

single nucleotide polymorphisms. Plans are currently underway to analyze more samples from the southeast regions of Bangladesh to evaluate this hypothesis.

ACKNOWLEDGMENTS

This work was supported by a base grant to the California National Primate Research Center from National Center for Research Resources at the National Institutes of Health (RR000169-48 to D.G. S.), and RR005090 and RR025781 (D.G.S.) and the National Institute of Allergy and Infectious Diseases (R01 AI078229; R01AI078229-03S1; R03 AI064865 to L.J.-E.). We are grateful to the Forest Department of Bangladesh for their permission and constant support during fieldwork and to the authority of the Wildlife Rescue Center (WRC) of the Department of Zoology, Jahangirnagar University for their logistics during sample collection. The first author thanks Dr. Yoshi Kawamoto and the Primate Research Institute (PRI), Kyoto University, Japan for providing valuable training in molecular genetics.

REFERENCES

- Abegg C, Thierry B. Macaque evolution and dispersal in insular south-east Asia. *Biological Journal of the Linnean Society*. 2002; 75:555–576.
- Akhtar, S. Monkey performers in Bangladesh: monkey population, health care, human–monkey interaction and socio-economic condition of the monkey owners [MPhil dissertation]. Department of Zoology, Jahangirnagar University; Savar, Dhaka, Bangladesh: 2014.
- Barlow AC, Smith JL, Ahmad IU, et al. Female tiger *Panthera tigris* home range size in the Bangladesh Sundar-bans: the value of this mangrove ecosystem for the species' conservation. *Oryx*. 2011; 45:125–128.
- Bensasson D, Zhang D-X, Hartle DL, et al. Mitochondrial pseudogenes: evolution's misplaced witnesses. *Trends in Ecology & Evolution*. 2001; 16:314–321. [PubMed: 11369110]
- Bhuiyan MA, Rakib M, Takashi K, et al. Regulation of Brahmaputra–Jamuna river around Jamuna bridge site, Bangladesh: geoenvironmental impacts. *Journal of Water Resource and Protection*. 2010; 2:123–130.
- Collura RV, Stewart CB. Insertions and duplications of mtDNA in the nuclear genomes of Old World monkeys and hominoids. *Nature*. 1995; 378:485–489. [PubMed: 7477403]
- Deinard A, Smith DG. Phylogenetic relationships among the macaques: evidence from the nuclear locus NRAMP1. *Journal of Human Evolution*. 2001; 41:45–59. [PubMed: 11414773]
- Delson, E. Fossil macaques, phyletic relationships and a scenario of deployment. Van Nostrand Reinhold; New York, NY: 1980. p. 10-30.
- Engel GA, Small CT, Soliven K, et al. Zoonotic simian foamy virus in Bangladesh reflects diverse patterns of transmission and co-infection. *Emerging Microbes & Infections*. 2013; 2:e58. [PubMed: 26038489]
- Eudey, AA. Pleistocene glacial phenomena and the evolution of Asian macaques.. In: Lindburg, DG., editor. *The macaques: studies in ecology, behavior and evolution*. Van Nostrand Reinhold; New York, USA: 1980. p. 52-83.
- Feeroz MM. Species diversity and population density of non-human primates in north-east and south-east of Bangladesh. *Ecoprint*. 2001; 8:53–57.
- Feeroz MM, Hasan K, Hamada Y, Kawamoto Y. STR polymorphism of mtDNA D-loop in rhesus macaques of Bangladesh. *Primates*. 2008; 49:69–72. [PubMed: 17530354]
- Feeroz, MM.; Hasan, MK.; Khan, MMH. Rema-Kalenga wildlife sanctuary. Vol. I. BioTrack. Arannayk Foundation; Bangladesh: 2011. Biodiversity of protected areas of Bangladesh..
- Feeroz MM, Soliven K, Small CT, et al. Population dynamics of rhesus macaques and associated foamy virus in Bangladesh. *Emerging Microbes & Infections*. 2013; 2:e29. [PubMed: 26038465]
- Ferguson B, Street SL, Wright H, et al. Single nucleotide polymorphisms (SNPs) distinguish Indian-origin and Chinese-origin rhesus macaques (*Macaca mulatta*). *BMC Genomics*. 2007; 8:43. [PubMed: 17286860]
- Fooden J. Provisional classification and key to living species of macaques (primates:Macaca). *Folia Primatologica*. 1976; 25:225–236.

- Fooden J. Taxonomy and evolution of the Sinica group of macaques. VI. Interspecific comparisons and synthesis. *Fieldiana Zoology*. 1988; 45:1–44.
- Fooden J. Comparative review of fascicularis-group species of macaques (primates: *Macaca*). *Fieldiana Zoology*. 2006; 107:1–43.
- Hasan, MK. Hollock gibbon of Bangladesh: status distribution and ecology [MSc dissertation]. Department of Zoology, Jahangirnagar University; Savar, Dhaka, Bangladesh: 2003.
- Hasan MK, Aziz MA, Alam SR, et al. Distribution of rhesus macaques (*Macaca mulatta*) in Bangladesh: inter- population variation in group size and composition. *Primate Conservation*. 2013; 26:125–132.
- Hernandez RD, Hubisz MJ, Wheeler D, et al. Genetic variation reveals diametric demographic histories and patterns of linkage disequilibrium for Chinese and Indian rhesus macaques. *Science*. 2007; 316:240–243. [PubMed: 17431170]
- Islam, M.; Ameen, M.; Nishat, A. Red book of threatened mammals of Bangladesh. IUCN; Dhaka: 2000.
- Islam N, Jamal AA, Chowdhury AM. *Banglapedia: national encyclopedia of Bangladesh*. Asiatic Society of Bangladesh. 2003; 2:415.
- Jones-Engel L, Engel GA, Heidrich J, et al. Temple monkeys and health implications of commensalism, Kathmandu, Nepal. *Emerging Infectious Diseases*. 2006; 12:900–906. [PubMed: 16707044]
- Khan, MAR. *Wildlife of Bangladesh: a checklist*. University of Dhaka; Dhaka: 1982.
- Khan, MMH. *Tigers in the mangroves: research and conservation of the tiger in the Sundarbans of Bangladesh*. Arannayk Foundation Bangladesh; Dhaka, Bangladesh: 2011.
- Kyes RC, Jones-Engel L, Chalise MK, et al. Genetic characterization of rhesus macaques (*Macaca mulatta*) in Nepal. *American Journal of Primatology*. 2006; 68:445–455. [PubMed: 16541441]
- Melnick DJ, Kidd KK. Genetic and evolutionary relationships among Asian macaques. *International Journal of Primatology*. 1985; 6:123–160.
- Melnick DJ, Hoelzer GA, Absher R, Ashley MV. mtDNA diversity in rhesus monkeys reveals overestimates of divergence time and paraphyly with neighboring species. *Molecular Biology and Evolution*. 1993; 10:282–295. [PubMed: 8487631]
- Ramachandran S, Deshpande O, Roseman CC, et al. Support from the relationship of genetic distance and geographic distance in human populations for a serial founder effect originating in Africa. *Proceedings of the National Academy of Sciences of the USA*. 2005; 102:15942–15947. [PubMed: 16243969]
- Smith DG, McDonough J. Mitochondrial DNA variation in Chinese and Indian rhesus macaques (*Macaca mulatta*). *American Journal of Primatology*. 2005; 65:1–25. [PubMed: 15645455]
- Stanford, CB. *The capped langur in Bangladesh: behavioral ecology and reproductive tactics*. Vol. 26. Karger Publishers; New York, USA: 1991.
- Stevison LS, Kohn MH. Divergence population genetics analysis of hybridization between 2457 rhesus and cynomolgus macaques. *Molecular Ecology*. 2009; 18:2457–2475. [PubMed: 19457196]
- Sussan, E.; Sollas, J.; Sollas, HBC. *The face of the earth*. Clarendon Press; Oxford, London: 1904.
- Tosi AJ, Morales JC, Melnick DJ. Paternal, maternal, and biparental molecular markers provide unique windows onto the evolutionary history of macaque monkeys. *Evolution*. 2003; 57:1419–1435. [PubMed: 12894949]

Fig. 1.

Map showing four sub-populations and sampling sites. Color dots indicate sampling sites in each sub-population (pink, sky blue, and yellow dots indicate the central, eastern, and southwestern sub-populations, respectively). The deep green color in the map indicates existing forests and the light green color indicates continuous forest connecting the northeast and southeast sectors of the eastern sub-population through India. Numbers indicate corresponding local populations of rhesus macaques sampled as follows: 1. Sadhana, 2. Dhamrai, 3. Narayanganj, 4. Bormi, 5. Madhupur, 6. Satchari, 7. Kalenga, 8. Fenchuganj, 9. Chashnipeer, 10. Syed Jahan, 11. Malnichara, 12. Khadimnagar, 13. Haripur, 14. Jaintapur, 15. Chandpur, 16. Chunati, 17. Fashiakhali, 18. Dulahazara, 19. Charmuguria, 20. Kolargaon, 21. Naria, 22. Nandanshar, 23. Kartikpur, 24. Wajirpur, and 25. Karamjal, Sundarbans. Areas bounded by a red line represent the three sub-populations of rhesus macaques in Bangladesh separated by river barriers. Sampling site 5 lies just on the western side of the main channel of the Brahmaputra River before it course was shifted westward by the 1,762 earthquake.

Fig. 2.

Map of the geographic range of *Macaca mulatta*; pink dots are the sampling locations in Bangladesh and origin of mtDNA sequences used for comparison. Locations 1–4 in India (Kashmir, New Delhi, Uttar Pradesh, and Lucknow, respectively), 5 in Nepal (Kathmunda), 6–9 in Bangladesh (northeast, central, southwest, and southeast, respectively), 10 in Myanmar (Yangon), 11–15 in China (China West: 11 and 12, Sichuan and Kunming, respectively; China South: 13 and 14, Guangxi and Guangdong, respectively; China East: 15, Suzhou), 16 in Vietnam [Smith & McDonough, 2005].

Fig. 3.

Median-joining haplotype network rooted with a *Macaca sylvanus* sequence illustrating three different haplogroups. Colors of the circles represent haplotypes from specific sub-populations of rhesus macaques in Bangladesh using the color codes cited in Figure 1 and the dark blue represents the *M. sylvanus* sequence, the root of the network. Sizes of the circles indicate the number of samples of that particular haplotype.

Fig. 4. Neighbor-joining (NJ) tree of rhesus macaque haplotypes showing five sub-clusters. Color bars represent the three sub-populations of rhesus macaques in Bangladesh using the color codes cited in Figures 1 and 2. Capital letters on the nodes correspond to nodes in Figure 5.

Fig. 5. Relationship between genetic distance and geographic distance among the rhesus macaques of Bangladesh. The red box identifies all samples from the southeastern region of Bangladesh. Note that the correlation between genetic and geographic distances is not statistically significant when the samples from the southeast region are not included.

Fig. 6. Neighbor-joining tree based on 762 bp sequences of mitochondrial DNA of rhesus from regional populations within their natural range. The size of the base of each triangle is proportional to sample size. Note that the oldest haplotypes are from Bangladesh and that most of the Bangladesh samples cluster with the Ind-2 haplotypes from India reflecting a westward direction of gene flow.

TABLE I

Sampling Sites With the Number of Recorded Haplotypes

Sampling sites	GPS coordination	No. of samples	No. of haplotypes	Haplotype names
Central sub-population				
1. Sadhana	N 23°42.192' E 90°25.477'	8	2	BR36, BR37
2. Dhamrai	N 23°55.07' E 90°12.42'	5	2	BR34, BR35
3. Narayanganj	N 23°36.903' E 90°30.716'	8	4	BR1, BR5, BR38, BR39
4. Bormi	N 24°14.588' E 90°31.391'	5	3	BR26, BR27, BR28
5. Madhupur	N 24°41.193, E90°08.569	7	1	BR23
Eastern sub-population				
6. Satchari	N 24°07.414 E 91°26.56.66'	7	5	BR9, BR10, BR11, BR43, BR45
7. Kalenga	N 24°09.54' E 91°37.32'	4	2	BR31, BR33
8. Fenchuganj	N 24°39.543' E 91°58.553'	1	1	BR31
9. Chashnipeer	N 24°54.535' E 91°52.6563'	5	2	BR16, BR17
10. Syed Jahan	N 24°55.745' E 91°52.572'	7	4	BR17, BR20, BR21, BR22
11. Malnichara	N 24°56.251' E 91°52.074'	2	2	BR18, BR31
12. Khadimnagar	N 24°57.438' E 91°55.979'	1	1	BR17
13. Haripur	N 24°58.054' E 92°01.580'	1	1	BR19
14. Jaintapur	N 25°05.930' E 92°07.768'	1	1	BR14
15. Chandpur	N 23°13.696' E 90°38.543'	6	2	BR1, BR7
16. Chunati	N 21°55.497' E 92°3.496'	4	3	BR3, BR41, BR42
17. Fashiakhali	N 21°40.45' E 92°04.49'	2	2	BR44, BR46
18. Dulahazara	N 21°40.084' E 92°4.788'	1	1	BR40
Southwest sub-population				
19. Charmuguria	N 23°10.249' E 90°10.036'	5	3	BR8, BR12, BR13
20. Kolargaon	N 23°16.276' E 90°28.608'	2	1	BR1
21. Naria	N 23°18.317' E 90°24.713'	2	1	BR1
22. Nandanshar	N 23°17.868' E 90°28.633'	2	1	BR1
23. Kartikpur	N 23°17.732' E 90°28.737'	6	3	BR1, BR2, BR4
24. Wajirpur	N 22°49.261' E 90°15.037'	4	2	BR29, BR30
25. Karamjal, SB	N 22°25.457' E 89°35.647'	9	5	BR6, BR15, BR24, BR25, BR32