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SNP based genetic characterization of the Tulane National Primate Research Center's (TNPRC) conventional and specific pathogen-free (SPF) Rhesus Macaque (*Macaca mulatta*) populations

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Abstract

Background—The rhesus macaque is an important biomedical model organism and the Tulane National Primate Research Center (TNPRC) has one of the largest rhesus macaque breeding colonies in the US.

Methods—SNP profiles from 3,266 rhesus macaques were used to examine the TNPRC colony genetic composition over time and across conventional or SPF animals of Chinese and Indian ancestry.

Results—Chinese origin animals were the least genetically diverse and the most inbred; however, since their derivation from their conventional forebearers, neither the Chinese nor the Indian SPF animals exhibit any significant loss of genetic diversity or differentiation.

Conclusions—The TNPRC colony managers have successfully minimized loss in genetic variation across generations. Although founder effects and bottlenecks among the Indian animals have been successfully curtailed, the Chinese subpopulation still show some influences from these events. Several genetic management recommendations to maximize genetic diversity and minimize inbreeding among the Chinese subpopulation have been included.

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Keywords

Genetic management; Single nucleotide polymorphisms (SNPs); Population genetics; Genetic structure; Genetic composition

Introduction

Rhesus macaques (*Macaca mulatta*) have a close evolutionary proximity to humans, which is one of the primary reasons why this species is widely used as a biomedical research model for studying infectious diseases and immunology (including AIDS research), neuroscience, reproductive biology, vaccine development and regenerative medicine.^{1–8} The Tulane National Primate Research Center (TNPRC) is among the largest of the seven National Primate Research Centers (NPRCs) in the United States that breed rhesus macaques for research. In 1976 the Food and Drug Administration (FDA) established a rhesus monkey breeding colony at TNPRC, which became the foundation for the current colony. There were 22 half-acre corrals stocked with approximately 50–60 young adult, wild-born rhesus from India with a sex ratio of one male to nine females. Although their genetic relatedness was not known, it was assumed that this original population consisted of largely unrelated individuals.

Following an export ban by the Indian government in 1978, smaller numbers of founders from other domestic primate centers and commercial vendors were introduced into the colony for breeding purposes and the enclosures were either modified or increased in numbers to accommodate the population growth and to implement various management strategies. Early in 1988, rhesus macaques from China were introduced into the colony; however, interbreeding between animals of Chinese and Indian origin was not a part of the breeding management plan and is thought to have not occurred. In 2001, the TNPRC created its specific pathogen-free (SPF) program to meet an increasing national demand for SPF animals for AIDS research. Conventional status (nonSPF) animals of Chinese and Indian ancestries were used to derive the current Chinese and Indian SPF breeding colony. Currently, there are approximately 3176 Indian and 450 Chinese SPF animals at the TNPRC. Although conventional animals are no longer being bred at this facility, it still holds about 40 non-SPF animals that are used for SPF derivation from aging and established disease phenotype colonies.

The TNPRC colony's breeding strategy has been described previously.⁹ Young males are removed from their natal groups at puberty (approximately 3.5yrs of age) and placed into peer groups for at least two years before being introduced to a non-related group of females. When resident breeding age males need to be replaced either due to reduced production or daughters having reached sexual maturity, they are removed from the group a few months prior to breeding season. An appropriate number of new breeding age males who are unrelated to the females in the social group are then introduced during breeding season.

A key measure of the success of any breeding system is the demonstration that the system has been able to maintain an appropriate level of genetic diversity and structure. Careful genetic management is essential to both facilitate the production of genetically characterized

animals for research and to conserve their genetic diversity. In addition to parameters such as observed and expected heterozygosity, allele numbers and inbreeding coefficients can be used to monitor the genetic diversity of the research stock, while matrices such as mean kinship scores and genome uniqueness can be calculated to identify genetically valuable animals for assignment into breeding groups.^{10–13} However, calculation of such values depends on detailed and accurate multigenerational pedigree information and while a pedigree is currently being developed for the TNPRC colony, it has not yet reached the generational depth to facilitate such calculations.

Assessing genetic diversity can, for example, be accomplished by different approaches such as whole genome sequencing, which at present represents considerable challenges and requires substantial financial resources for large colonies.^{14,15} A more convenient approach is the use of single nucleotide polymorphisms (SNPs) and, indeed, several groups have reported the discovery of large numbers of SNPs in the macaque genome.^{16–19} Moreover, a recent study in which whole genome data were generated from 132 rhesus macaque across all US primate centers has revealed that rhesus appear to exhibit a larger single nucleotide variation than either humans or most other nonhuman primates for which such data exist.²⁰

The identification of high variability among SNPs in a species that is critical for biomedical research is significant because of the likelihood that it will lead to the discovery of new models for human genetic diseases.^{21,22} Additionally, SNPs can be a valuable tool for calculating population genetic parameters to facilitate genetic management of breeding colonies. To that end, a 96-SNP genetic management panel was developed for parentage testing and for characterizing rhesus macaque colony genetics.²³ The present study utilized that panel of 96 SNPs to genotype the rhesus macaques at the TNPRC in order to assess the genetic composition of founders and subsequent generations of this colony.

Methods and Materials

Humane Care Guidelines

All procedures followed the Guidelines for the Care and Use of Laboratory Animals of the National Research Council and the TNPRC Standard Operating Protocols (SOPs). The TNPRC is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Experimental protocols were approved by the Tulane University Institutional Animal Care and Use Committee (IACUC) before implementation.

Experimental Methods

As described previously the panel of 96 SNPs used in this study was derived from a preliminary batch of 277 candidate SNPs based on two criteria: 1) a minimum estimate of 30% minor allele frequency in both the Chinese and Indian rhesus macaques; and 2) high levels of heterozygosity (e.g., between 0.4 and 0.5).²⁴ If the SNPs satisfied these requirements, the expectation is that these loci would be ideal for calculating gene diversity, genetic subdivision and average inbreeding coefficient, estimates that are relevant for population genetic and pedigree studies among animals of both Chinese and Indian origins.^{17,18,21,24}

The Illumina, Inc. (San Diego, CA) BeadXpress Assay, BeadStudio Reader, and GoldenGate™ Platform were used to genotype 3,266 rhesus macaque DNA samples from the TNPRC using this panel of 96 SNPs. The sample set included animals from the conventional and SPF breeding groups that were born between 1990 and 2014. The study animals included those presumed to be pure Indian or pure Chinese based on current colony records. The SNP genotyping results were evaluated using quality control metrics and genotyping failure rates as described elsewhere.^{18,21,24} Those SNPs that exhibited GenTrain quality scores below 0.65 or had >5% missing data were eliminated from further analysis. Only animals which exhibited at least 90% complete genotypes with GenCall scores >0.65 across the 96 SNPs were included in subsequent population genetic analyses.

To investigate diachronic changes in genetic composition among the entire dataset, the colony wide samples were subdivided into five 5-year periods that reflected the year in which an animal was born (Tables 1 and 2). To compare and contrast the genetic compositions of the conventional and SPF animals of Chinese and Indian ancestry, the study animals were classified into four categories that reflected their SPF status as well as their ancestry (Tables 1 and 3).

Arlequin v3.5.1.3 was used to calculate observed heterozygosity (OH), gene diversity (expected heterozygosity, EH), and minor allele frequencies (MAF).²⁵ This software has previously been used to compute Wright's F-statistics: FIS (inbreeding coefficient), FST (the total coancestry or population subdivision coefficient), and FIT (the total fixation coefficient) to assess the genetic composition of rhesus monkeys at the California National Primate Research Center (CNPRC).^{10,11,26}

Results

The 197 samples with less than 90% profiles were excluded from further analysis. All 96 SNPs exhibited two alternate alleles (i.e., were biallelic) and none of these loci yielded an equilibrium probability lower than 0.01 across the colony-wide data set including the 4 subpopulations of SPF and non-SPF Chinese and Indian animals.

Estimates of OH, EH, MAF and FIS are provided in Table 1. Longitudinally, values of OH and EH ranged from 0.46 to 0.47 and 0.39 to 0.43 from 1990 to 2014 with an average of 0.46 and 0.41, respectively (Table 1). OH and EH estimates were 0.39 and 0.47 and between 0.40 and 0.47, respectively, among the four groups studied with average values of 0.43 and 0.44. The Chinese SPF animals recorded the lowest estimates of heterozygosity among the four groups studied while the Indian SPF animals exhibited an equal amount of heterozygosity to that of the conventional Indian animals; the Indian subpopulation was consistently more genetically diverse than the Chinese subpopulation based on their OH and EH values. MAF values in the TNPRC colony remained constant through time, however when the Chinese and Indian animals were analyzed separately, MAF values were significantly different between them (i.e., 0.33 in the Chinese animals vs 0.40 in the Indian animals).

FIS ranged from 0.02 to 0.04 across time with an average of 0.03. The conventional Chinese exhibited the highest inbreeding levels (0.06) while the Chinese SPF animals showed much lower levels of inbreeding (0.04). Inbreeding due to consanguineous matings in the Indian subpopulation was either nonexistent (FIS of conventional subpopulation is approximately 0) or negligible (FIS of SPF subpopulation is approximately 0.01). The especially low value of F_{ST} ($\ll 0.01$) based on diachronic estimates was unexpected and reflects remarkable genetic homogeneity among the samples from the time periods. When the dataset was divided according to animal ancestry and SPF status, a high mean F_{ST} of 0.12 was measured between Chinese and Indian animals regardless of their SPF status. However, both SPF Chinese and Indian animals have not differentiated from their respective conventional forbearers ($F_{ST} \ll 0.01$) (Table 2).

Across time as well as among the different subpopulations of SPF and conventional animals, the genetic structure of the SPF colony from 1990 to 2014, as measured by the overall F -statistic estimates ranged from low to moderate. Across time, colonywide FIS, F_{ST} , and FIT of 0.03, 0 and 0.03, respectively, were generated, while computations from the four subpopulations based on ancestry and SPF status generated FIS, F_{ST} and FIT of 0.007, 0.06 and 0.06, respectively. Based on the AMOVA, the bulk of the genetic variation exists at the individual levels (94–96%) while 4–6% of the variation was observed among the different subpopulations studied.

Discussion

Genetic management of captive bred rhesus macaques based on population genetic approaches provides an invaluable insight into these animals' genetic variability and suitability as models for biomedical experimentation. Population genetic parameters can reveal important population dynamics within a captive colony and validate management approaches to promote colony genetic diversity, minimize inbreeding among animals and prevent genetic differentiation among enclosures and across generations. For instance, a study based on short tandem repeats (STRs) revealed that the derivation of SPF animals at the CNPRC resulted in SPF populations that have maintained a relatively high degree of genetic diversity; however, successive generations have become significantly diverged from their respective conventional founders and to a lesser degree from each other.¹⁰ This divergence resulted in the loss of several rare STR alleles including some at MHC-linked loci. Unlike STRs, SNP-based analyses cannot be used to detect loss of alleles over time due to the absence of multiple alternative alleles per locus. However, deviations between values of observed (OH) and expected heterozygosity (EH) can be used to detect the presence or absence of inbreeding among colony animals.²⁷

In this study, longitudinal estimates of mean heterozygosity (OH and EH) from the TNPRC rhesus colony showed that OH was consistently significantly greater than EH ($p < 0.5$) suggesting isolate-breaking effects due to a colony management strategy that not only promotes gene flow across generations of animals, but also minimizes inbreeding levels. OH and EH values however, were approximately commensurate (0.43 vs 0.44) when the respective conventional and SPF animals of Chinese and Indian subpopulations were compared. This occurrence in combination with low F_{ST} values between the SPF animals

and their respective conventional ancestors of either geographic origin indicate low levels of genetic differentiation during SPF derivation.¹⁰ This further demonstrates the effectiveness of the TNPRC's strategy to derive and expand its SPF animals from the conventional breeding stock without compromising genetic diversity. While this observation also presumptively attests to the pure blood status of all animals that compose the SPF and conventional subpopulations, a large scale admixture analysis would be necessary to confirm any hybridity within the TNPRC colony that may have resulted from inadvertent interbreeding between the Chinese and Indian animals.^{10,28,29}

Limiting opportunities for consanguineous matings by replacing resident males with unrelated males into social groups during breeding season has very likely played a central role in suppressing inbreeding levels among colony animals.⁹ While inbreeding is almost non-existent in the Indian animals (FIS = 1%), the Chinese animals of both SPF and conventional status consistently exhibited higher FIS estimates (i.e., about 4% among the SPF animals and about 6% among the conventional animals, respectively) and lower heterozygosity (approximately 7% lower). The lower genetic diversity (based on OH, EH and MAF) among the TNPRC Chinese animals is not unique to this colony as it has also been observed at the CNPRC where the Chinese subpopulation is reportedly less genetically heterogeneous than the Indian subpopulation.^{10,23,28,30}

Founder effects and genetic bottlenecks during the introduction of Chinese rhesus macaques into US breeding facilities may have shaped their current genetic composition. For example, the Chinese animals at the CNPRC descended from founders obtained from only a handful of breeding centers in China including Kunming, Shanghai and Suzhou, even though there are far more primate breeding facilities throughout China.³¹ This has been strongly suspected to have created a genetic bottleneck among the CNPRC Chinese animals as reflected by their low levels of heterozygosity (0.22) and MAF (0.15) which incidently are also much lower than the heterozygosity and MAF values exhibited by the TNPRC Chinese animals in the present study, i.e. 0.40 and 0.33, respectively^{10,11,27} Furthermore, unlike the TNPRC Indian animals which were obtained from multiple North American sources, the TNPRC Chinese subpopulation was established from founders imported directly from China.⁹

Due to a ban on the capture of wild monkeys, rhesus macaques from China are bred in breeding facilities for domestic use as well as for export purposes.³² As such many of the wild-caught Chinese founders which were originally used to establish the breeding facilities within China were probably related to each other. Finding close relatives among male-breeders is not unusual when social groups for captive breeding are established.²⁸ Therefore, diversity loss due to another bottleneck event probably occurred when animals from the Chinese facilities were later introduced into the TNPRC colony. This probably explains the higher inbreeding coefficient (FIS=0.06) among the conventional Chinese animals, which is approximately one and a half times greater than their SPF descendants (FIS=0.04).

The Indian colony at the TNPRC also represents a much larger subpopulation than the Chinese subpopulation. In larger populations, founder contributions do not vary significantly across generations.^{12,27} Genetic diversity is also often correlated with effective population

size or N_e , which is not only influenced by the number of male breeders but also these males' variance in reproductive success (RS).¹² The introduction of Chinese animals into the TNPRC colony happened much more recently than that of Indian animals (1988 vs 1976); therefore, the effects of the TNPRC's colony management strategy to promote genetic diversity may not have affected the Chinese subpopulation to the degree it has the Indian subpopulation. This combination of factors could have also fostered the higher levels of relatedness and lower genetic variation among the Chinese conventional and SPF descendants. Based on the heterozygosity and F-statistic estimates from the TNPRC SNP data, our results demonstrate that the breeding colony management has been successful in preventing genetic variation from dissipating or differentiating over time. In addition to the conserved genetic variation between the founders and the birth cohorts in this colony, the significant divide (F_{ST}) between Chinese and Indian animals of both conventional and SPF status seem to indicate that any admixture of domestically bred rhesus macaques of Chinese and Indian origins have not occurred.

While the present study's results reveal that the TNPRC colony managers have successfully managed to stem inbreeding levels due to consanguinity among its Indian animals, the Chinese subpopulation still exhibit some low level inbreeding from founder effects and genetic bottlenecks when the Chinese animals were first introduced into the TNPRC colony. To counter the potential loss of genetic diversity, colony management practices should continue to focus on increased gene flow and reduced inbreeding by increasing male animal numbers within the Chinese social groups, removing males with the highest RS, equalizing RS between males and females and promoting cross-fostering infants from other social groups. With the tools of genetic management, such as the panel of SNP markers used in this study, the loss of genetic diversity due to inbreeding and subdivisions, and the associated deleterious phenotypic effects can be minimized. Efforts are underway at the TNPRC as well as other primate centers to obtain more comprehensive genome data on animals and with the expected decline in cost it is likely that such information will not only play a key role in how rhesus colonies are managed in the future, but also provide more insight into the genetic structure of captive rhesus colonies.

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Table 1

Estimates of observed (OH), expected heterozygosity (EH), inbreeding coefficient (FIS), and minor allele frequency (MAF). N is the sample size for each study group.

	N	OH	EH	FIS	MAF
Entire colony-longitudinal					
1990–1994	88	0.46	0.43	0.04	0.41
1995–1999	221	0.47	0.40	0.02	0.41
2000–2004	858	0.46	0.39	0.03	0.41
2005–2009	1230	0.46	0.40	0.03	0.41
2010–2014	638	0.46	0.41	0.03	0.41
<i>Average</i>	<i>607</i>	<i>0.46</i>	<i>0.41</i>	<i>0.03</i>	<i>0.41</i>
Total	3035				
Conventional Indian	443	0.47	0.47	0.00	0.40
SPF Indian	2279	0.47	0.47	0.01	0.40
Conventional Chinese	84	0.40	0.41	0.06	0.33
SPF Chinese	229	0.39	0.40	0.04	0.33
<i>Average</i>	<i>759</i>	<i>0.43</i>	<i>0.44</i>	<i>0.03</i>	<i>0.37</i>
Total	3035				

Table 2

Pairwise FST among the 5 year periods.

	1990–1994	1995–1999	2000–2004	2005–2009
1995–1999	0.0002			
2000–2004	0.0001	0.0005		
2005–2009	0.0004	0.0003	0.0002	
2010–2014	0.0007	0.0007	0.0007	0.0005

Table 3

Pairwise FST among the different subpopulations.

	Chinese SPF	Chinese Conventional	Indian SPF
Chinese Conventional	0.0020		
Indian SPF	0.1195	0.1049	
Indian Conventional	0.1227	0.1053	0.0006