Title

Genetic Characterization of a Captive Colony of Pigtailed Macaques (Macaca nemestrina).

Journal

Journal of the American Association for Laboratory Animal Science, 56(4)

ISSN

1559-6109

Authors

Zhang, Xinjun
Kanthaswamy, Sree
Trask, Jessica S
et al.

Publication Date

2017-07-01

Peer reviewed

Journal of the American Association for Laboratory Animal Science
Copyright 2017
by the American Association for Laboratory Animal Science

Vol 56, No 4
July 2017
Pages 390–395

Genetic Characterization of a Captive Colony of Pigtailed Macaques (*Macaca nemestrina*)

Xinjun Zhang,[1] Sree Kanthaswamy,[2] Jessica S Trask,[1] Jillian Ng,[1] Robert F Oldt,[2] Joseph L Mankowski,[3,4] Robert J Adams,[3] and David G Smith[1,*]

Effective colony management is critical to guarantee the availability of captive NHP as subjects for biomedical research. Pigtailed macaques (*Macaca nemestrina*) are an important model for the study of human and nonhuman primate diseases and behavior. Johns Hopkins University hosts one of the largest captive colonies of pigtailed macaques in the United States. In this study, we used 56 single-nucleotide polymorphisms (SNP) to characterize this population of pigtailed macaques, understand their population structure, and assess the effectiveness of their colony management. The results demonstrate that the colony has maintained a high level of genetic diversity, with no loss of heterozygosity since its origin, and low levels of inbreeding and genetic subdivision.

Abbreviations: AMOVA, analysis of molecular variance; EH, expected heterozygosity; F_e, founder equivalent value; F_g, founder genome equivalent value; F_{ST}, amount of genetic variation among the populations in AMOVA; OH, observed heterozygosity; SNP, single-nucleotide polymorphism

NHP are widely bred in colonies in the United States as models for biomedical and biobehavioral research.[14] In addition to the 2 more commonly used macaque species—rhesus macaques (*Macaca mulatta*) and cynomolgus macaques (also called long-tailed macaques or crab-eating macaques, *M. fascicularis*)—pigtailed macaques (*M. nemestrina*) have been widely used as models for studies of infectious diseases (including HIV infection and AIDS),[3,4,12,16,30] immunology,[32,33] neuroscience,[17,29] pathology,[2,15] and behavior.[5,13,37] Because of their outgroup evolutionary relationship with rhesus and cynomolgus macaques, pigtailed macaques also serve as a valuable comparison group in comparative evolutionary studies, such as those focusing on MHC compatibility[21] and its relationship to infectious disease susceptibility.

Wild pigtailed macaques are widely distributed—from northeastern India, Bangladesh, southern China, and Vietnam to Peninsular Malaysia, Borneo, and Sumatra.[8,10] Although once considered to be the same species (*M. nemestrina*), southern pigtailed macaques (*M. nemestrina*, the assumed species of the pigtailed macaques that are currently captively bred in the United States) and northern pigtailed macaques (*M. leonine*) were later classified as separate species based on morphology[8] and mitochondrial DNA evidence.[31] The overlap between the geographic distributions of these 2 species in the wild with those of other macaque species, such as cynomolgus macaques,[9] suggests that admixture between *M. nemestrina* and *M. leonina* or other species is possible. However, no evidence has yet confirmed admixture, and small amounts of admixture in the early phylogenetic history of each species could result in little observable phenotypic difference.

Captive colonies are often derived from a small number of founders that, together with the typically high variance in male reproductive success[34] and low sex ratio in most captive macaque colonies, could lead to a small effective population size.[41] Genetic heterogeneity is lost at a rate inversely proportional to the effective population size, with a resultant loss of fitness over subsequent generations due to inbreeding depression and genetic subdivision.[20] Consequently, such colonies should be genetically monitored so that genetic management procedures can be implemented to minimize loss of colony fitness and maximize genetic heterogeneity.[1] In addition to its use as a tool for colony management, population genetic analysis of colonies—such as estimates of the degree and distribution of genetic heterogeneity,[27] coefficients of kinship and inbreeding, admixture, and identification of the ancestral origin of the founders of the colony—is valuable in assessing the suitability of animals as models for the study of particular diseases.[35]

A previous study used 18 short tandem repeat markers (STR) to genotype the captive pigtailed macaque colony at the Washington National Primate Research Center.[19] The results revealed that the Washington colony of pigtailed macaques exhibits greater genetic heterogeneity than most captive colonies of rhesus macaques.[19] Through careful genetic management, the colony has successfully maintained maximum founder representation and low levels of inbreeding.

A second colony of 158 pigtailed macaques, whose genetic structure has not been reported previously, has been maintained at Johns Hopkins University since 1999. This colony was established by the introduction of approximately 100 pigtailed macaques acquired from the following breeding facilities throughout the United States during the year indicated in parentheses: the Tulane National Primate Research Center (1999), Covance (2003), Laboratory Animal Breeders and Services of Virginia (2003), Pennsylvania State University–Hershey (2005), the Yerkes National Primate Research Center (2006), Alpha Genesis (formerly known as Laboratory Animal Breeders and Services of Virginia, 2007), the Washington National Primate

Received: 26 Aug 2016. Revision requested: 21 Dec 2016. Accepted: 10 Mar 2017.
[1]Department of Anthropology and California National Primate Research Center, University of California Davis, Davis, California; [2]School of Mathematical and Natural Sciences, Arizona State University, Glendale, Arizona; and Departments of [3]Molecular and Comparative Pathobiology and [4]Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland.
[*]Corresponding author. Email: dgsmith@ucdavis.edu

Research Center (2008), and the New Iberia Primate Research Center (2009). The initial colony was established using pigtailed macaques obtained from 3 of the 7 listed breeding facilities (Tulane, Covance, Laboratory Animal Breeders and Services of Virginia), which produced the first offspring cohort, born between 2003 and 2008. However, founding yearlings from the 4 other breeding facilities (Pennsylvania State University–Hershey, Yerkes National Primate Research Center, Washington National Primate Research Center, and New Iberia Primate Research Center) were introduced to the colony between 2005 and 2009. Yearlings began breeding by 2008, when pairings of sires and dams were organized to randomize breeding and avoid inbreeding. Between 2003 and 2012, the founding colony experienced 200 new births, from which all surviving females and a few males were retained as future breeders.

Surviving macaques from the founder group that gave birth to the first cohort of offspring (born in 2003) included approximately 6 males and 21 females. During subsequent years, the number of breeders varied from 8 to 13 males and 33 to 70 females, with an average effective population size of approximately 32 animals (an estimate based on the assumption that males and females become sexually mature at 4.5 and 3.5 y of age, respectively).

Given the heterogeneous origins of the colony, we expected a relatively high level of genetic heterogeneity, as well as a significant level of population structure due to strong genetic subdivision (although this genetic subdivision can be mitigated by careful selective breeding). Moreover, because macaques of different ages were acquired from different breeding facilities and introduced at different times during the development of the colony, significant changes in the genetic structure of the initial offspring cohorts might have occurred during the early stages of the colony's development.

In addition to short tandem repeats, single-nucleotide polymorphisms (SNP) have been used in population genetics with increasing frequency. In mammalian genomes, SNP are more abundant than STR, showing closer linkage to sites of interest, and are easily adaptable to high-throughput analysis with high accuracy in genotype calls.[18,26] Due to advances in DNA sequencing technologies, the development and analysis of SNP markers are now less costly than those of STR and can be applied to both captive and wild animals of all primate species.[18,40] In the current study, we used SNP to genotype the pigtailed macaques at Johns Hopkins University to characterize the genetic structure and heterogeneity of the founders and subsequent birth cohorts of this captive colony.

Materials and Methods

We identified the SNP in the current study by modifying a methodology developed for SNP identification in rhesus macaques.[39] We selected 25 unrelated pigtailed macaques from the colony for this study, because unrelated individuals capture most of the genetic diversity in an entire colony and provide for the most efficient discovery of the most informative loci.[38] DNA samples from the 25 macaques were normalized to a concentration of 60 ng/µL and digested with *Hin*fI. Fragments between 200 and 500 bp in length were harvested from each animal (Invitrogen E-Gel System, Life Technologies, Carlsbad, CA) and pooled in equal volumes for construction of a paired-end reduced representation library (Paired-End Library Construction Kit, Illumina, San Diego, CA). The library was quantified by using PCR analysis (model 7300 Fast Real-time PCR System with Sequence Detection Software, version 1.4.0.25, Applied Biosystems, Foster City, CA), and its size range confirmed (model 2100 Bioanalyzer, Agilent Biotechnologies, Santa Clara, CA). The library (both forward and reverse strands) was sequenced (HiSEquation 2000, Illumina) by the UCD Genome Center (Davis, CA).

The forward and reverse strands of each read were paired and mapped to the rhesus macaque genome by using the program BWA (Burrows–Wheeler Aligner),[23] excluding fragments with low mapping scores, and the program SAMtools (version 1.4)[24] was used to identify nucleotide positions that were polymorphic among the pigtailed macaques. The 50 different chromosomes included in the sample pool ensured that most overlapping fragments in the library derived from different animals, providing an accurate estimate of the frequencies of the alleles at polymorphic sites. SNP with PHRED scores above 25 and minor allele frequencies greater than 0.05 were considered true polymorphisms. We identified 5013 SNP and selected the 192 that had the highest minor allele frequencies, were approximately equidistantly distributed across the genome, and were representative of all autosomes to construct a pigtailed macaque–specific SNP panel. Flanking primer sequences for the 192 SNP were submitted to Fluidigm (South San Francisco, CA) for assay design, and those suitable for multiplexing with the highest estimated minor allele frequency were selected for construction of the 96-SNP panel. The locations of these SNP are available upon request.

During this study, we genotyped 162 pigtailed macaques from the colony at Johns Hopkins University (Figure 1). Among the 162 macaques, 58 were originally imported between 1999 and 2007 from the 7 primate facilities mentioned earlier and were considered the founding group. Subsequently, 104 macaques were born into the colony between 2003 and 2013. Detailed information on the 162 members of the colony is provided as Supplemental Material Table 1. For the purpose of evaluating genetic heterogeneity and inbreeding, the 162 macaques were divided into the following 3 populations: the founder population, which comprised 58 breeders, a cohort of 38 macaques born between 2003 and 2008 and a cohort of 66 animals born between 2009 and 2013.

DNA from the 162 animals was extracted from whole blood (QIAamp DNA Mini Extraction Kit, Qiagen, Valencia, CA) according to the manufacturer's protocol. The DNA was quantified fluorometrically (Qubit 1.0, Thermo Fisher Scientific, Waltham, MA) and the concentration standardized to 60 ng/µL. The DNA samples were aliquoted into two 96-well plates and sent to the UC Davis Genome Center for genotyping with the 96-SNP assay described earlier and using the Fluidigm EP1 system. The SNP genotypes were called by using Fluidigm's SNP Genotyping Analysis software, and only macaques and markers for which data were at least 90% complete were used for subsequent analysis.

Arlequin version 3.5.2.2[7] was used to calculate observed heterozygosity (OH), gene diversity (or expected heterozygosity [EH]) and Hardy–Weinberg equilibrium for the founders and 2003–2008 and 2009–2013 birth cohorts. Unbiased Hardy–Weinberg equilibrium *P* values were calculated by using the Markov Chain Monte Carlo approach with 10,000 iterations per batch, and departures from Hardy–Weinberg equilibrium were determined at the 0.05 significance level by using the Fisher Exact Test.[11]

Arlequin was also used to compute F statistics, including the Wright inbreeding coefficient,[42] to test for the combined loss of heterozygosity due to nonrandom mating within the colony, to conduct analysis of molecular variance (AMOVA) and to estimate population-specific F_{ST}, which corresponds to

Type	Breeding facility	No. of macaques	Total
Founders	Pennsylvania State University–Hershey	4	58
	Covance Primate Research Center	4	
	Laboratory Animal Breeders and Services of Virginia/Alpha Genesis	13	
	New Iberia Primate Research Center	6	
	Tulane University	1	
	Yerkes National Primate Research Center	14	
	Washington National Primate Research Center	16	
Offspring	Born 2003–2008	38	104
	Born 2009–2013	66	
Total no. of pigtailed macaques			162

Figure 1. Sources and numbers of pigtailed macaques included in this study.

the genetic variation among populations in AMOVA, and mean pairwise F_{ST}. The statistical significance of pairwise F_{ST} estimates was tested by using a probability distribution constructed from permutations of 1000 iterations with Bonferroni corrections for multiple comparisons.

Because all animals in the birth cohorts have documented records of dam and sire from the original imported founder animals, this colony also represents a pedigreed colony. Therefore, founder representation analysis[22] can be used to characterize and monitor its genetic diversity. The Python programming language (Python Language Reference, version 3.5.2, http://www.python.org) was used to implement the calculation of founder equivalent (F_e) and founder genome-equivalent (F_g) metrics for the colony. F_e is the number of founders sufficient to produce the observed level of genetic diversity of a colony if all founders contributed equally to subsequent generations, whereas F_g is the number of equally contributing founders required to produce a population with the observed level of genetic diversity without the loss of any founder alleles. The average inbreeding coefficient and the expected level of heterozygosity under random mating can be approximated as $1/(2 \times F_g)$ and $1 - [1/(2 \times F_g)]$, respectively. An F_g value of approximately 20 is regarded as sufficient for retaining at least 90% of the genetic heterogeneity of a group of founders over multiple generations of breeding.[22]

Results

A total of 80 of the 162 pigtailed macaques evaluated yielded SNP genotype data that were at least 90% complete, representing 34 founder macaques, 22 animals from the 2003–2008 birth cohort, and 24 progeny in the 2009–2013 cohort. Of the 96 SNP markers used, 56 were both in Hardy–Weinberg equilibrium and generated 90% complete genotype data for distance computation, with a 10% level of missing data allowed, and were therefore used for population genetic analyses of these 80 macaques. The estimates of effective population size, OH and EH obtained by using these loci and averaged across the 3 populations are shown in Table 1. The OH and EH (mean ± 1 SD) were 0.38 ± 0.12 and 0.40 ± 0.12 for the founder population, 0.37 ± 0.14 and 0.39 ± 0.11 for the 2003–2008 birth cohort, and 0.39 ± 0.14 and 0.39 ± 0.11 for the 2009–2013 cohort. Thus, all 3 cohorts of the colony exhibited highly consistent levels of genetic diversity, with OH being slightly lower than EH.

Figure 2 presents the pairwise F_{ST} for the 3 populations. The pairwise F_{ST} between the founders and both the 2003–2008 (F_{ST}, 0.0102; $P = 0.0541 \pm 0.0201$) and the 2009–2013 (F_{ST}, 0.0084; $P = 0.0270 \pm 0.0194$) birth cohorts were statistically significant but that between the 2 birth cohorts themselves ($F_{ST} = –0.0006$, interpreted as 0.0000; $P = 0.4955 \pm 0.0563$) was not.

The AMOVA results illustrating the source of genetic variation in the colony are given in Table 2. The majority of the total genetic variation occurred within individuals (95.5%), with only a small fraction among individuals within each population (3.9%), and less than 1% was from the differences among the 3 groups. For the entire colony overall, the average inbreeding coefficient of the individual relative to its subpopulation was 0.039, average genetic distance (fixation index) was 0.006, and average inbreeding coefficient of the individual relative to the total population was 0.045. These values were consistent with the AMOVA results.

The founder representation analysis revealed that the F_e value was 18.8909 and that F_g was 17.9502. Although suggestive of an imbalance of contribution among the 58 founders of the colony, these results are consistent with the high level of genetic diversity in this pedigreed colony, illustrated by our analysis of OH and EH.

Discussion

The 2 birth cohorts in the studied colony maintained approximately the same level of heterozygosity as in the founder population. The moderate excess in expected heterozygosity compared with observed heterozygosity and the low F statistics demonstrate that this captive pigtailed macaque colony has experienced low levels of inbreeding and genetic subdivision. The multiple origins of the colony's founders and their varied times of and ages at introduction to the colony might have otherwise fostered genetic subdivision, whereas the limited number of male founders, together with a high variance in male reproductive success, could have fostered significant levels of inbreeding. For example, founders from Tulane, Covance, and Laboratory Animal Breeders and Services of Virginia were introduced in 2003 as adults, whereas those from other breeding facilities were introduced as yearlings (Penn State University–Hershey and Alpha Genesis in 2005, Yerkes National Primate Research Center in 2007, Washington National Primate Research Center in 2008, and the New Iberia Primate Research Center in 2009). Thus, only 9 of the 57 offspring born into the colony before 2007, and 46 of 99 offspring born between 2010 and 2012 (46%) were sired by founding males from breeding facilities other than Covance and Laboratory Animal Breeders and Services of Virginia. The first births to founders from the Washington National Primate Research Center did not occur until spring 2011. Consequently, comparisons of the founder population with both subsequent birth cohorts yielded statistically significant F_{ST} values, probably predominantly due to nonrandom mating among the founding males. Nevertheless, the average F_{ST} value among all 3 subsets of the colony was less than 1% ($F_{ST} = 0.006$).

Table 1. Estimates of genetic diversity based on 56 polymorphic biallelic SNP loci[a]

	Population founders			Population 2008			Population 2013		
	Ng	OH	EH	Ng	OH	EH	Ng	OH	EH
SNP_at_chr1_103540071	64	0.50	0.48	44	0.50	0.38	46	0.43	0.43
SNP_at_chr1_208459566	66	0.48	0.47	44	0.27	0.30	48	0.42	0.42
SNP_at_chr1_37712808	68	0.32	0.28	44	0.41	0.38	48	0.38	0.36
SNP_at_chr10_19985876	68	0.32	0.47	42	0.24	0.40	48	0.38	0.36
SNP_at_chr10_70888540	64	0.63	0.51	44	0.55	0.51	44	0.41	0.46
SNP_at_chr11_127262148	68	0.29	0.33	40	0.20	0.26	48	0.25	0.22
SNP_at_chr11_131784245	64	0.41	0.47	44	0.32	0.46	48	0.50	0.51
SNP_at_chr11_133364699	68	0.47	0.40	44	0.32	0.27	44	0.32	0.33
SNP_at_chr11_13495302	68	0.47	0.50	44	0.50	0.50	48	0.58	0.50
SNP_at_chr12_87384570	68	0.18	0.21	44	0.32	0.27	48	0.25	0.22
SNP_at_chr13_123686128	68	0.53	0.51	44	0.27	0.47	48	0.63	0.49
SNP_at_chr13_129094301	64	0.38	0.51	44	0.50	0.51	48	0.46	0.49
SNP_at_chr13_71810391	68	0.47	0.49	44	0.50	0.46	48	0.25	0.38
SNP_at_chr13_99077025	68	0.35	0.37	44	0.32	0.49	48	0.58	0.42
SNP_at_chr14_72397772	68	0.15	0.14	44	0.14	0.13	48	0.13	0.19
SNP_at_chr15_100507671	66	0.15	0.24	44	0.18	0.17	48	0.33	0.28
SNP_at_chr17_31395904	68	0.35	0.40	44	0.41	0.38	48	0.25	0.28
SNP_at_chr17_40357735	68	0.44	0.45	44	0.41	0.43	48	0.38	0.51
SNP_at_chr17_83523698	68	0.26	0.23	44	0.41	0.43	48	0.29	0.25
SNP_at_chr17_8499221	68	0.24	0.33	44	0.36	0.30	48	0.33	0.38
SNP_at_chr18_38429101	66	0.52	0.44	42	0.33	0.28	48	0.29	0.36
SNP_at_chr18_8571147	68	0.09	0.09	44	0.09	0.09	46	0.17	0.16
SNP_at_chr19_11710500	64	0.41	0.42	40	0.15	0.30	48	0.54	0.44
SNP_at_chr19_15333422	66	0.48	0.50	44	0.45	0.47	46	0.48	0.48
SNP_at_chr19_34550001	66	0.42	0.47	42	0.43	0.51	46	0.48	0.41
SNP_at_chr19_35418302	68	0.21	0.23	44	0.09	0.09	48	0.08	0.08
SNP_at_chr19_46026311	68	0.53	0.51	44	0.64	0.47	48	0.50	0.48
SNP_at_chr2_158497496	68	0.53	0.48	44	0.14	0.27	48	0.33	0.34
SNP_at_chr2_17995033	68	0.26	0.28	44	0.27	0.41	48	0.38	0.44
SNP_at_chr2_189405197	68	0.56	0.49	44	0.50	0.49	48	0.54	0.47
SNP_at_chr2_27858342	68	0.35	0.44	44	0.14	0.27	48	0.29	0.36
SNP_at_chr3_102510551	66	0.39	0.48	44	0.27	0.41	48	0.50	0.38
SNP_at_chr3_7867608	66	0.42	0.40	42	0.48	0.42	46	0.39	0.45
SNP_at_chr4_155304831	66	0.33	0.32	44	0.32	0.46	48	0.46	0.36
SNP_at_chr4_166810860	68	0.18	0.21	44	0.18	0.24	48	0.13	0.12
SNP_at_chr4_32206907	68	0.53	0.51	42	0.43	0.47	44	0.55	0.51
SNP_at_chr4_36702115	68	0.41	0.48	44	0.41	0.49	48	0.67	0.51
SNP_at_chr4_57514610	68	0.32	0.28	44	0.50	0.49	48	0.33	0.38
SNP_at_chr5_133642495	66	0.27	0.24	44	0.45	0.36	44	0.64	0.44
SNP_at_chr5_19779811	68	0.29	0.33	44	0.50	0.46	48	0.38	0.49
SNP_at_chr5_2743999	66	0.30	0.30	42	0.29	0.32	48	0.38	0.31
SNP_at_chr5_37775354	68	0.44	0.41	44	0.27	0.30	48	0.25	0.34
SNP_at_chr5_7238901	68	0.47	0.51	44	0.64	0.51	48	0.50	0.51
SNP_at_chr6_20611630	68	0.38	0.49	44	0.45	0.44	48	0.33	0.28
SNP_at_chr6_22071127	68	0.44	0.41	44	0.45	0.47	48	0.21	0.31
SNP_at_chr6_43263616	66	0.12	0.12	44	0.27	0.30	44	0.27	0.41
SNP_at_chr7_129740844	68	0.35	0.37	44	0.55	0.49	48	0.46	0.47
SNP_at_chr7_159682555	68	0.47	0.50	44	0.36	0.51	48	0.46	0.51
SNP_at_chr8_123762504	68	0.41	0.46	44	0.50	0.46	48	0.25	0.45
SNP_at_chr8_132364582	68	0.35	0.50	44	0.36	0.47	48	0.33	0.50
SNP_at_chr8_135827564	68	0.41	0.49	44	0.27	0.49	48	0.38	0.49
SNP_at_chr8_30071562	68	0.38	0.43	42	0.24	0.40	48	0.38	0.31
SNP_at_chr9_120784637	68	0.38	0.41	44	0.41	0.46	48	0.29	0.40
SNP_at_chr9_17142726	68	0.56	0.49	42	0.48	0.51	44	0.68	0.51

Table 1. Continued

	Population founders			Population 2008			Population 2013		
	Ng	OH	EH	Ng	OH	EH	Ng	OH	EH
SNP_at_chr9_65958336	66	0.45	0.51	44	0.55	0.49	48	0.42	0.50
SNP_at_chr9_7491920	68	0.41	0.49	44	0.59	0.49	48	0.58	0.48
Mean	67.21	0.38	0.40	43.57	0.37	0.39	47.39	0.39	0.39
1 SD	1.30	0.12	0.12	0.99	0.14	0.11	1.32	0.14	0.11

EH, expected heterozygosity; Ng, number of genotypes; OH, observed heterozygosity
[a]These 56 SNP loci are among the 96 that provided 90% complete data and are both polymorphic and in Hardy–Weinberg equilibrium ($P = 0.05$).

Pairwise F_{ST}

	Founders	2003–2008	2009–2013
Founders	0		
2003–2008	0.01016	0	
2009–2013	0.00845	–0.00063	0

F_{ST} *P* values

	Founders	2003–2008	2009–2013
Founders	*		
2003–2008	0.05405 ± 0.0201	*	
2009–2013	0.02703 ± 0.0194	0.49550 ± 0.0563	*

Matrix of significant F_{ST} values (threshold: $P = 0.05$)

	Founders	2003–2008	2009–2013
Founders		+	+
2003–2008	+		—
2009–2013	+	—	

Figure 2. Genetic variation among the 3 populations of pigtailed macaques (F_{ST}).

Table 2. Analysis of molecular variation (AMOVA)

Source of variation	Sum of squares	Variation component	Percentage of variation
Among populations	29.904	0.06688	0.60111
Among macaques within population	874.82	0.43502	3.90995
Within macaques	839.5	10.62411	95.48894
Total	1744.223	11.12602	

Correspondingly, the variance in the reproductive success of founder males declined from 98.6 to 29.3 between the springs of 2010 (11 males) and 2012 (12 males) and produced roughly equal numbers of offspring (110 and 99, respectively). This situation resulted in a significant increase in the effective population size of the colony, reducing the opportunity for inbreeding of the 2 offspring cohorts (Wright inbreeding coefficient, <5%).

For finite-sized colonies in which the sexual maturity of related animals varies—even those that use paired matings—consanguineous mating and loss of heterozygosity is eventually unavoidable[19] but can be minimized through improved management strategies. Such strategies include stricter surveillance, increasing effective population size by increasing the number of males in the groups or by removing males with the highest reproductive success to balance founder representation and cross-fostering infants from other groups to increase gene flow.[36] Our present results suggest that the studied pigtailed macaque colony has been managed successfully to prevent the loss of genetic variation.

In the current study, 96 SNP were selected for genotyping, but only 56 yielded meaningful results. This relatively low percentage of useful SNP markers suggests that the criteria we used to select SNP for pigtailed macaques may need to be reevaluated and improved. Alternatively, other informative genetic markers, especially short tandem repeats that have been used in other genetic studies of macaques (including pigtailed macaques),[19,28] could be used in parallel with SNP to provide more detailed results of population genetics.

Despite the conserved genetic variation between the founders and birth cohorts in the focus colony, the results do not eliminate the concern of potential admixture of domestically bred pigtailed macaques (most likely *M. nemestrina*) with *M. leonina*. For example, studies in rhesus macaques have shown that, compared with Indian-origin macaques, Chinese rhesus macaques are less susceptible to SIV and are therefore unsuitable as models for HIV research.[6,16,25,35] Because pigtailed macaques are used as animal models in multiple biomedical studies, a clearer understanding of their genetic ancestry and whether admixture is present is necessary to determine their suitability as disease models.

Population genetic analysis and the characterization of pigtailed macaques in captive colonies provide important and valuable insights into their genetic variability and suitability as animal models. Here we report that the pigtailed macaques at Johns Hopkins University exhibit a sustained level of genetic heterogeneity and low levels of inbreeding and genetic subdivision, thus providing an excellent example of well-conceived and well-executed genetic management procedures.

Acknowledgments

This study was supported by the California National Primate Research Center base grant (No. RR000169-48), by grants RR005090 and RR025781 to DGS from the National Center for Research Resources of NIH, and by P40 OD0131117 (NIH) to RJA. This research adhered to the American Society of Primatologists principles for the ethical treatment of NHP, and the animals used have been managed in compliance with IACUC regulations or in accordance with the NIH guidelines or the US Department of Agriculture regulations prescribing the humane care and use of laboratory animals. We thank the Molecular Anthropology Laboratory (MAL) of Department of Anthropology at UC Davis for providing facility support for this study and the Genome Center of UC Davis for performing genotyping assay. We also thank Johns Hopkins University for providing the samples used in this study.

References

1. **Ballou J, Ralls K.** 1982. Inbreeding and juvenile mortality in small populations of ungulates: a detailed analysis. Biol Conserv **24:**239–272.
2. **Baskin CR, García-Sastre A, Tumpey TM, Bielefeldt-Ohmann H, Carter VS, Nistal-Villán E, Katze MG.** 2004. Integration of clinical data, pathology, and cDNA microarrays in influenza virus-infected pigtailed macaques (*Macaca nemestrina*). J Virol **78:**10420–10432.

3. **Batten CJ, De Rose R, Wilson KM, Agy MB, Chea S, Stratov I, Montefiori DC, Kent SJ.** 2006. Comparative evaluation of simian, simian–human, and human immunodeficiency virus infection in pigtail macaque (*Macaca nemestrina*) model. AIDS Res Hum Retroviruses **22:**580–588.
4. **Beck SE, Kelly KM, Queen SE, Adams RJ, Zink MC, Tarwater PM, Mankowski JL.** 2015. Macaque species susceptibility to simian immunodeficiency virus: increased incidence of SIV central nervous system disease in pigtailed macaques versus rhesus macaques. J Neurovirol **21:**148–158.
5. **Bellanca RU, Crockett CM.** 2002. Factors predicting increased incidence of abnormal behavior in male pigtailed macaques. Am J Primatol **58:**57–69.
6. **Cohen J.** 2000. AIDS research. Vaccine studies stymied by shortage of animals. Science **287:**959–960.
7. **Excoffier L, Laval G, Schneider S.** 2007. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform Online **1:**47–50.
8. **Fooden J.** 1975.Taxonomy and evolution of liontail and pigtail macaques (Primates: *Cercopithecidae*), vol 67. Chicago (IL): Field Museum of Natural History.
9. **Fooden J.** 1996. Zoogeography of Vietnamese primates. Int J Primatol **17:**845–899.
10. **Groves C.** 2001.Primate taxonomy (Smithsonian series in comparative evolutionary biology). Washington (DC): Smithsonian Institutional Press.
11. **Guo SW, Thompson EA.** 1992. Performing the exact test of Hardy–Weinberg proportion for multiple alleles. Biometrics **48:**361–372.
12. **Hatziioannou T, Ambrose Z, Chung NPY, Piatak M Jr, Yuan F, Trubey CM, Coalter V, Kiser R, Schneider D, Smedley J, Pung R, Gathuka M, Estes JD, Veazey RS, KewalRamani VN, Lifson JD, Bieniasz PD.**2009. A macaque model of HIV1 infection. Proc Natl Acad Sci USA **106:**4425–4429.
13. **Heath-Lange S, Ha JC, Sackett GP.** 1999. Behavioral measurement of temperament in male nursery-raised infant macaques and baboons. Am J Primatol **47:**43–50.
14. **Held JR.** 1981. Breeding and use of nonhuman primates in the USA: breeding and use of nonhuman primates in the USA. Int J Study Anim Probl **2:**27–37.
15. **McClure HM, Anderson DC, Fultz PN, Ansari AA, Lockwood E, Brodie A.** 1989. Spectrum of disease in macaque monkeys chronically infected with SIV/SMM. Vet Immunol Immunopathol **21:**13–24.
16. **Joag SV, Stephens EB, Galbreath D, Zhu GW, Li Z, Foresman L, Zhao LJ, Pinson DM, Narayan O.** 1995. Simian immunodeficiency virus SIVmac chimeric virus whose *env* gene was derived from SIV-encephalitic brain is macrophage-tropic but not neurovirulent. J Virol **69:**1367–1369.
17. **Juul SE, Aylward E, Richards T, McPherson RJ, Kuratani J, Burbacher TM.** 2007. Prenatal cord clamping in newborn *Macaca nemestrina*: a model of perinatal asphyxia. Dev Neurosci **29:**311–320.
18. **Kanthaswamy S, Gill L, Satkoski J, Goyal V, Malladi V, Kou A, Basuta K, Sarkisyan L, George D, Smith DG.** 2009. Development of a Chinese–Indian hybrid (Chindian) rhesus macaque colony at the California National Primate Research Center by introgression. J Med Primatol **38:**86–96.
19. **Kanthaswamy S, Ng J, Penedo MCT, Ward T, Smith DG, Ha JC.** 2012. Population genetics of the Washington National Primate Research Center's (WaNPRC) captive pigtailed macaque (*Macaca nemestrina*) population. Am J Primatol **74:**1017–1027.
20. **Kanthaswamy S, Von Dollen A, Kurushima JD, Alminas O, Rogers J, Ferguson B, Lerche N, Allen PC, Smith DG.** 2006. Microsatellite markers for standardized genetic management of captive colonies of rhesus macaques (*Macaca mulatta*). Am J Primatol **68:**73–95.
21. **Knapp LA, Ha JC, Sackett GP.** 1996. Parental MHC antigen sharing and pregnancy wastage in captive pigtailed macaques. J Reprod Immunol **32:**73–88.
22. **Lacy RC.** 1989. Analysis of founder representation in pedigrees: founder equivalents and founder genome equivalents. Zoo Biol **8:**111–123.
23. **Li H, Durbin R.** 2009. Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics **25:**1754–1760.
24. **Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R.** 2009. The sequence alignment–map format and SAMtools. Bioinformatics **25:**2078–2079.
25. **Ling B, Veazey RS, Luckay A, Penedo C, Xu K, Lifson JD, Marx PA.** 2002. SIVmac pathogenesis in rhesus macaques of Chinese and Indian origin compared with primary HIV infections in humans. AIDS **16:**1489–1496.
26. **Malhi RS, Trask JS, Shattuck M, Johnson J, Chakraborty D, Kanthaswamy S, Ramakrishnan U, Smith DG.** 2011. Genotyping single nucleotide polymorphisms (SNPs) across species in Old World monkeys. Am J Primatol **73:**1031–1040.
27. **Melnick DJ.** 1987. The genetic consequences of primate social organization: a review of macaques, baboons, and vervet monkeys. Genetica **73:**117–135.
28. **Morin PA, Kanthaswamy S, Smith DG.** 1997. Simple sequence repeat (SSR) polymorphisms for colony management and population genetics in rhesus macaques (*Macaca mulatta*). Am J Primatol **42:**199–213.
29. **Rausell E, Bickford L, Manger PR, Woods TM, Jones EG.** 1998. Extensive divergence and convergence in the thalamocortical projection to monkey somatosensory cortex. J Neurosci **18:**4216–4232.
30. **Rey-Cuille MA, Hu SL.** 2001. Conserved CXCR4 usage and enhanced replicative capacity of HIV2/287, an isolate highly pathogenic in *Macaca nemestrina*. AIDS **15:**2349–2357.
31. **Rosenblum LL, Supriatna J, Melnick DJ.** 1997. Phylogeographic analysis of pigtail macaque populations (*Macaca nemestrina*) inferred from mitochondrial DNA. Am J Phys Anthropol **104:**35–45.
32. **Shaulov A, Murali-Krishna K.** 2008. CD8 T-cell expansion and memory differentiation are facilitated by simultaneous and sustained exposure to antigenic and inflammatory milieu. J Immunol **180:**1131–1138.
33. **Shibata R, Igarashi T, Haigwood N, Buckler-White A, Ogert R, Ross W, Willey R, Cho MW, Martin MA.** 1999. Neutralizing antibody directed against the HIV1 envelope glycoprotein can completely block HIV1–SIV chimeric virus infections of macaque monkeys. Nat Med **5:**204–210.
34. **Smith DG.** 1994. Genetic heterogeneity in 5 captive specific pathogen-free groups of rhesus macaques. Lab Anim Sci **44:**200–210.
35. **Smith DG, McDonough J.** 2005. Mitochondrial DNA variation in Chinese and Indian rhesus macaques (*Macaca mulatta*). Am J Primatol **65:**1–25.
36. **Smith S.** 1986. Infant cross-fostering in rhesus monkeys (*Macaca mulatta*): a procedure for the long-term management of captive populations. Am J Primatol **11:**229–237.
37. **Sussman A, Ha J.** 2011. Developmental and cross-situational stability in infant pigtailed macaque temperament. Dev Psychol **47:**781–791.
38. **Trask JA, Malhi RS, Kanthaswamy S, Johnson J, Garnica WT, Malladi VS, Smith DG.** 2011. The effect of SNP discovery method and sample size on estimation of population genetic data for Chinese and Indian rhesus macaques (*Macaca mulatta*). Primates 52:129–138.
39. **Trask JS, Garnica WT, Kanthaswamy S, Malhi RS, Smith DG.** 2011. 4040 SNPs for genomic analysis in the rhesus macaque (*Macaca mulatta*). Genomics **98:**352–358.
40. **Williams LM, Ma X, Boyko AR, Bustamante CD, Oleksiak MF.** 2010. SNP identification, verification, and utility for population genetics in a nonmodel genus. BMC Genet **11:**1–14.
41. **Wright S.** 1931. Evolution in mendelian populations. Genetics **16:**97–159.
42. **Wright S.** 1978. Evolution and the genetics of populations: variability within and among natural populations, vol 4. Chicago (IL): University of Chicago Press.