The Population Genetic Composition of Conventional and SPF Colonies of Rhesus Macaques (Macaca mulatta) at the Caribbean Primate Research Center

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The SPF breeding program at the Caribbean Primate Research Center supplies Indian-origin rhesus macaques of known genetic and virologic background for biomedical research. In this study, population genetic analyses using 14 short tandem-repeat sequences showed that the SPF colony has remained genetically homogenous over time, with sufficient amounts of heterozygosity and minimal stratification from its founders. Intergenerational studies indicated that an average of 7 alleles have been retained, inbreeding levels have remained low, and the degree of Indian ancestry is one of the highest among several national primate research centers. The relative low genetic diversity in the free-ranging population as well as in the captive SPF and conventional colonies when compared with that of other primate centers indicates that the free-ranging population, from which the captive-colony animals were derived, has experienced significant founder effects and genetic drift during the years after its establishment. This study supports the historical origin of the free-ranging population and confirms the high value of this resource for biomedical research. Current genetic diversity levels within the SPF colony can be ensured with the practice of colony management approaches such as equalizing male:female ratios in each SPF breeding group and increasing breeding group sizes. Introducing new Indian-origin macaques from other captive colonies might help to maximize the genetic diversity of the breeding stock. Furthermore, genetic estimates must be used to rank breeders according to their genetic value or their genome uniqueness to increase founder-genome representation and curb future genetic bottlenecks and allele loss.

Abbreviations: EH, expected heterozygosity; OH, observed heterozygosity; STR, short tandem-repeat sequence

Primate resources at the Caribbean Primate Research Center consist of free-ranging rhesus macaques on the island of Cayo Santiago (n = 1321, 31%) as well as the Cayo Santiago-derived rhesus macaques housed in a captive setting at the Sabana Seca Field Station (n = 2941, 69%). The Cayo Santiago population is the oldest free-ranging colony maintained under minimal human intervention,1 and since its establishment in 1938 when 409 founders from India were released onto Cayo Santiago, the island population has remained a closed colony. Replacements since the introduction of the original stock have been recruited exclusively through births. Since 1995, an average of 139 ± 97 (mean ± 1 SD) rhesus macaques has been translocated annually from Cayo Santiago to the field station to stabilize the island’s population size. No animals are returned to Cayo Santiago once they are removed from the island.12

With all its founders derived exclusively from annual roundups in Cayo Santiago, the captive colonies at the field station currently consist of approximately 924 macaques of conventional health status as well as 2083 specific-pathogen-free (SPF) animals and SPF candidates. These animals are housed in different outdoor enclosures at the field station, including 40 field corrals and 205 corncrib cages. The captive macaques at the field station allow for more invasive and manipulative types of biomedical studies than do their free-ranging counterparts in Cayo Santiago. Most of the macaques at the field station—that is male and female adults, juveniles, and infants—are maintained in social groups in large outdoor field corrals, whereas the remaining animals are kept in harem breeding or juvenile peer groups in outdoor pens or in individual cages, depending on breeding requirements or their use in research protocols.

The conventional macaques at the field station are seropositive for B virus and STLV1 and seronegative for SIV and SRV-D.33 Currently, offspring from the conventional and SPF breeding stock at the field station jointly supply the Center’s SPF program. Established in 2000, the SPF colony at the field station is negative for B virus, SIV, SRV-D, and STLV1.32 The primary purpose of the Center’s SPF breeding program is to provide Indian-origin rhesus macaques of known genetic and virologic background to NIH-sponsored research on HIV–AIDS and other infectious agents, and surplus animals from this SPF program are made available for other biomedical research. Infants in the conventional and SPF colonies that are 6 mo old and weigh at least 1.5 kg are weaned from their dams and screened at the quarantine facility for recruitment into the SPF program. Once confirmed seronegative for all viruses, the immature macaques are formed into small SPF groups of 6 females to 1 male and are housed in corncrib cages adjacent to the quarantine facility.32
Different ancestral backgrounds and the inherent genetic variability of colony-raised outbred rhesus macaques influence how individual study animals respond to a particular experimental protocol, including drug testing and viral infection experiments. For example, differences in responses to SIV infection between Indian-origin macaques, such as those captive bred at the field station, and Chinese rhesus macaques have led to the greater use of Indian-origin animals as a rhesus model for research on SIV infection and AIDS.7,22,36 This recognition has resulted in the production of genetically characterized animals with known geographic origins and genealogy.15,20,21

Over the last 5 y, the field station has supplied research animals to more than 30 research institutions, including 6 international facilities, and these resources have facilitated collaborations with at least 35 investigators in the United States and elsewhere. Without diminishing their colony’s overall production goals, the colony managers at the field station intend to use the information generated in this study to facilitate the further development of defined pedigrees for formulating specific research objectives and to promote the conservation of allele diversity within candidate genes that govern phenotypes of adaptive and biomedical significance.

Materials and Methods

The 14 short tandem-repeat sequences (STR) used in this study have been reported elsewhere.14 STR typing was performed as described previously, and only the genotypes for the 3101 animals with complete STR profiles across all 14 loci were used for the population genetic analysis, resulting in a total of 1884 subjects from the free-ranging colony, 288 animals from the captive conventional colony, and 929 captive SPF macaques. We used Arlequin version 3.5.1.310 to calculate allele frequencies, observed heterozygosity (OH), and gene diversity (expected heterozygosity, EH). The same software was used to compute Wright’s F statistics—F_w (inbreeding coefficient), FST (the total coancestry or population subdivision coefficient), and F_T (the total fixation coefficient)—to test for the combined loss of heterozygosity due to nonrandom mating in the rhesus macaque population at the field station.

The identity of the age cohort (year of birth) as well as the generation to which an animal belonged was used to investigate diachronic changes in genetic composition among the study macaque. The generation to which an SPF macaque (n = 929) belongs was defined as 1 plus the maximum of the sire and dam generation numbers.11 Table 1 lists the number of samples tested per generation. Arlequin10 also was used to compute Weir and Cockerham pairwise FST values among age cohorts and among generations of animals, to assess the degree of divergence due to genetic drift. The significance of the pairwise FST estimates was determined from a probability distribution constructed from permutation tests (n = 100) with Bonferroni corrections for multiple comparisons.29

Structure 2.3.45,27 was used to illustrate the genetic composition of the SPF population. The software uses a Markov Chain Monte Carlo method to compute L(K), the posterior probability that the data fits the hypothesis of K genetically distinct groups. A training set comprising STR data from 177 pure Indian and 177 pure Chinese rhesus macaques from the California National Primate Center was used to determine the genetic structure among the SPF animals at the Sabana Seca Field Station. The animals from the California Center were used as references in this study because their ancestries were confirmed based on STR and single-nucleotide polymorphism data as well as animal importation documents and colony records.14,16,19 By using genotype data from 14 STR and a priori population information, mean and variance of log likelihoods and posterior probabilities for K-values of a maximum of 2 possible source populations, that is from India and China, respectively, were generated to characterize the genetic structure among the study macaques.14 All Structure runs were performed in sweeps of 500,000 iterations after a burn-in period of 100,000. In this analysis, allele frequencies were assumed to be correlated among populations and that, despite a priori assignment of an animal to a particular population, there is a high probability that it has ancestors in other source populations, due to admixture. All analyses were replicated 5 times, with each set of assumptions to assure that group assignments with the greatest probabilities were detected. The inferred value of K was the highest estimate of L(K) corresponding to the lowest run variance.8,27

Animals involved in this study were managed in compliance with IACUC regulations and with the NIH guidelines prescribing the humane care and use of laboratory animals. This research complies with the protocols approved by the IACUC (protocol numbers: 7890112 and 7890113) at the University of Puerto Rico and with the American Society of Primatologists Principles for the Ethical Treatment of Nonhuman Primates.2

Results

According to the assignment probabilities of the pure Chinese (more than 90% degree of Chinese ancestry) and pure Indian (less than 10% degree of Chinese ancestry) reference macaques,14,16,17 each of the free-ranging and captive macaques in the present study was estimated to be of predominantly Indian ancestry (greater than 98% degree of Indian ancestry). This outcome is in close agreement with colony records of their Indian-origin progenitors.

The average number of STR alleles and the average OH and expected EH of genotypes in each generation and year (based on the animals’ years of birth) are presented in Tables 1 and 2. Both the free-ranging (n = 1884) and the captive (n = 288) conventional populations exhibited comparable estimates of genetic variability (that is, number of STR alleles, OH, and EH) to those of the SPF colony (n = 929). Both tables reveal very slight fluctuations in the distribution of captive-conventional-wide STR heterozygosity in each generation and year of birth. Overall estimates of allele numbers among the 1217 captive conventional and SPF macaques show that an average of 7 alleles over time are present, and when effects from small sampling sizes (number of animals sampled in each generation) were ignored, alleles have been retained across generations. Both the OH and EH estimates among the (n = 1217) were slightly (about 6% and 9%, respectively) lower than estimates from the Indian reference animals (n = 177), and the estimated number of alleles for the study macaques was close to 42% lower than that estimated for the reference animals.

In addition, Table 1. presents estimates of inbreeding (FST) and genetic differentiation (or pairwise FST) among the free-ranging and captive conventional populations and the 4 subsequent generations of SPF animals, whereas Table 2. gives the annual FST values that were computed for the SPF colony. The free-ranging macaques showed a lower degree of inbreeding than did the captive conventional animals (FST = 0.0001 compared with 0.0086). However, the estimates of inbreeding coefficients across the entire SPF dataset suggest that the inbreeding levels at the colony have not decreased, ranging from −0.0005 to −0.03 (Table 1). Furthermore, pairwise differentiation (pairwise FST) over generations is minimal among the various study groups (FST = −0.0003 to 0.0159; Table 1). Table 2 illustrates the slight
Table 1. Cross-generational estimates of allele numbers, observed heterozygosity (OH), expected heterozygosity (EH), inbreeding ($F_{IS}$), and pairwise $F_{ST}$ (italics) among the study macaques, including animals from the Cayo Santiago source population (free-ranging macaques) and the captive conventional founders and their SPF descendants at Sabana Seca Field Station

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<tr>
<td>OH</td>
<td>0.71</td>
<td>0.71</td>
<td>0.71</td>
<td>0.72</td>
<td>0.71</td>
<td>0.71</td>
<td>0.71</td>
<td>0.71</td>
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<tr>
<td>EH</td>
<td>0.71</td>
<td>0.71</td>
<td>0.71</td>
<td>0.72</td>
<td>0.71</td>
<td>0.71</td>
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<tr>
<td>$F_{IS}$</td>
<td>0.0001</td>
<td>0.0086</td>
<td>−0.0042</td>
<td>−0.0005</td>
<td>−0.0013</td>
<td>−0.0265</td>
<td>−0.0081</td>
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</tr>
<tr>
<td>SPF generation 1</td>
<td>0.0044</td>
<td>−0.0003</td>
<td>−0.0002</td>
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<td>−0.0002</td>
<td>−0.0002</td>
<td>−0.0002</td>
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</tr>
<tr>
<td>SPF generation 2</td>
<td>0.0014</td>
<td>0.0011</td>
<td>0.0034</td>
<td>0.0004</td>
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<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td>SPF generation 3</td>
<td>0.0015</td>
<td>0.0159</td>
<td>0.01</td>
<td>0.0121</td>
<td>0.0084</td>
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<td>SPF generation 4</td>
<td>0.0106</td>
<td>—</td>
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All data are given as mean values.

Table 2. Annual changes in estimates of allele numbers, observed heterozygosity (OH), expected heterozygosity (EH), and inbreeding ($F_{IS}$)

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of alleles</th>
<th>OH</th>
<th>EH</th>
<th>$F_{IS}$</th>
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<tr>
<td>1995 (n = 2)</td>
<td>2.79</td>
<td>2.68</td>
<td>2.47</td>
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<tr>
<td>1996 (n = 4)</td>
<td>4.07</td>
<td>3.93</td>
<td>3.56</td>
<td>0.14</td>
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<td>1998 (n = 15)</td>
<td>5.43</td>
<td>5.26</td>
<td>4.95</td>
<td>−0.14</td>
</tr>
<tr>
<td>1999 (n = 22)</td>
<td>5.86</td>
<td>5.76</td>
<td>5.41</td>
<td>0.08</td>
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<tr>
<td>2000 (n = 57)</td>
<td>6.50</td>
<td>6.19</td>
<td>5.74</td>
<td>0.00</td>
</tr>
<tr>
<td>2001 (n = 113)</td>
<td>7.29</td>
<td>6.95</td>
<td>6.51</td>
<td>−0.01</td>
</tr>
<tr>
<td>2002 (n = 86)</td>
<td>7.29</td>
<td>6.87</td>
<td>6.53</td>
<td>0.03</td>
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<tr>
<td>2003 (n = 99)</td>
<td>7.07</td>
<td>6.77</td>
<td>6.39</td>
<td>−0.03</td>
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<tr>
<td>2004 (n = 110)</td>
<td>7.07</td>
<td>6.85</td>
<td>6.55</td>
<td>−0.02</td>
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<tr>
<td>2005 (n = 130)</td>
<td>6.93</td>
<td>6.69</td>
<td>6.28</td>
<td>−0.01</td>
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<td>2006 (n = 57)</td>
<td>6.57</td>
<td>6.35</td>
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<tr>
<td>2007 (n = 79)</td>
<td>6.86</td>
<td>6.45</td>
<td>6.06</td>
<td>−0.03</td>
</tr>
<tr>
<td>2008 (n = 143)</td>
<td>6.93</td>
<td>6.53</td>
<td>6.23</td>
<td>0.04</td>
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<tr>
<td>2009 (n = 11)</td>
<td>4.79</td>
<td>4.60</td>
<td>4.24</td>
<td>0.01</td>
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<tr>
<td>2010 (n = 1)</td>
<td>1.79</td>
<td>1.75</td>
<td>1.61</td>
<td>−0.05</td>
</tr>
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</table>

Mean: 5.81 5.58 5.23 0.00

$n$ is the sample size for each cohort.

All data are given as mean values.

Overall $F_{IS}$ among all annual age SPF cohorts is 0.04.

Discussion

The higher estimate of inbreeding in the captive conventional generation compared with the SPF colony at the Sabana Seca Field Station likely emerged because the conventional dataset contained many more closely related macaques when entire families (where a family is defined as all subjects that share the same parents) were included in the analysis.

Estimates of genetic variability among the free-ranging macaques on Cayo Santiago were lower than that of the reference Indian-origin animals. Consequently, the captive conventional and SPF macaques exhibited mean numbers of alleles and heterozygosity values that are about 60% and 94%, respectively, of the estimates generated from the reference Indian animals (Table 1). This result is consistent with findings from other previous cross-center comparisons where the macaques at the Sabana Seca Field Station showed STR-based OH and EH values that were about 9% lower than those at the California National Primate Research Center.22 SNP profiles from breeders from the Caribbean, Yerkes, and Southwest National Primate Research Centers indicated that the macaques at the Sabana Seca Field Station exhibited the lowest EH estimates—44% compared with a mean of 46% among the other 3 centers (range, 45% to 47%). A previous study4 reported a lack of protein variation among the Cayo Santiago-derived macaques compared with other macaque populations, suggesting that the island’s lower genetic heterogeneity may be evidence that its population, the sole source of the captive colonies at the field station, has experienced a decline in heterozygosity due to founder effects and genetic bottlenecks over its approximately 80-y history.

A founder effect occurs when a few members of an original population start a new colony, and the Cayo Santiago founders reportedly were trapped in 12 different districts around Lucknow, the capital city of Uttar Pradesh in India.6 Except for the original introduction of Indian origin animals into Cayo Santiago in the late 1930s,5,28 no other introductions of animals have occurred on the island.34 Despite the assumption that the original stock was probably outbred given that many of the animals were derived from separate breeding populations and therefore presumably to be unrelated,6 this single founder event is probably the initial genetic diversity-limiting factor in the colony at Sabana Seca Field Station. Most primate breeding facilities have experienced numerous introductions of new animals that were purchased from multiple breeding facilities (that is, sources) to replenish the genetic composition of their breeding stock.19 In addition, as recently as 2013, the California National Primate Research Center purchased macaques from Sabana Seca Field Station to introduce more Indian origin genes into its colony.
Genetic bottlenecks occur when a population’s size is reduced for at least one generation. Because genetic drift (or allelic drift) acts more quickly to reduce genetic variation in small populations relative to larger ones, undergoing a bottleneck can reduce a population’s genetic variation significantly, even if the bottleneck does not last for many generations. An episode of an extreme bottleneck event combined with strong genetic drift severely reduced rare mutations within the Mauritian cynomolgus macaque (Macaca fascicularis) population. Despite being introduced to Mauritius around 5 centuries ago, the time that has elapsed since this bottleneck period has been insufficient for the recovery of novel mutations in Mauritian cynomolgus macaques. As a consequence, based on whole-genome sequence evidence, Mauritian cynomolgus macaques show lower estimates of rare nonsynonymous mutations and exhibit a higher proportion of heterozygous nonsynonymous mutations to heterozygous synonymous mutations than do their more genetically diverse ancestral stock in the Malay Archipelago in Southeast Asia. The previously cited study’s findings are consistent with those of other authors, who attributed low STR allelic diversity to small founder representation, demographic bottlenecks, and subsequent rapid population expansions. These same forces appear to have affected the Cayo Santiago’s rhesus population since its establishment in the 1930s.

By estimating that approximately 90% of the rhesus macaques on Cayo Santiago descended from only 15 unrelated female progenitors that were alive in the mid-1950s, one author determined that at least one serious bottleneck has occurred. Varying accounts of low animal numbers due to conflicts, infanticide, disease, and occasionally inadequate food supplies on Cayo Santiago have been reported, including a low estimate of 70 macaques in 1956, which led to reduced genetic variation among the colony animals attributed to the restricted effective population size. By the late 1950s, regular provisioning stabilized animal numbers, but culling continued as needed. According to a previous report, potential sires in the years subsequent to the 1950s were probably not related to the 15 founder females and thus contributed to excess heterozygosity before the studies documenting bottlenecks were completed. Those early studies also concluded that declines in among group differentiation correlated with the timing of animal removal and rapid postremoval increases in animal numbers. In addition, the longitudinal serial attrition (by removal) and replacement (by birth) of macaques in the free-ranging island population may have shaped the genetic structure and composition of the conventional and SPF colonies at the Sabana Seca Field Station when it relied on animals removed from the island as replacements.

Presently, the breeding strategy at the Sabana Seca Field Station uses small breeding groups comprising 1 male and 6 female macaques to promote normal social interactions and maternal care and to minimize risks of exposure to the entire SPF colony should an animal seroconvert. However, this practice increases the potential for founder effects, genetic bottlenecks, and loss of rare alleles over time due to genetic drift, which can cause a substantial degree of genetic divergence between age cohorts and among individual animals. The direct comparison between the conventional and SPF animals at the Sabana Seca Field Station has provided a perspective on how the SPF colony has fared genetically since its derivation from its conventional forbearers. The present study shows that although the SPF colony remains genetically homogenous with sufficient amounts of heterozygosity, its allelic diversity is still much lower than those at other colonies, due to the colony’s distinct population history and management approaches.

In this study, periodic estimates of pairwise genetic differentiation among the SPF generations of animals suggest that the SPF macaques at the differentiated only slightly over time. Despite the low to moderate estimates, the FST values presented in Tables 1 and 2 reveal that the effect of genetic drift across age cohorts is about 3-fold greater than across generations. Lower pairwise FST estimates are indicative of greater overlaps in the frequencies of common alleles among generations than among the age cohorts of the animals compared. In addition, inbreeding levels have not accrued and allele numbers have remained consistent over time (Table 1). Elsewhere, rare alleles reportedly have gone extinct due to closed captive breeding programs. The loss of these rare alleles can potentially undermine the identification of associations between rare alleles and rare diseases. In addition, the structure analysis of the SPF population at Sabana Seca Field Station reflects a pure Indian stock, and the degree of Indian ancestry is among the highest that have been calculated across several major national primate centers.

Maintenance of current genetic diversity levels within the SPF colony can be bolstered by balancing the male:female ratio in each SPF breeding group to maximize the effective population size of the colony relative to its census size. Achieving this goal might require an increase in the breeding group size. When candidates are selected for configuring breeding groups (or removal from these groups), genetic estimates must be computed to rank them according to their genetic value, including their heterozygosity, inbreeding coefficients, mean kinship estimates (that is, the number of relatives the candidate will have in the breeding group), and genome uniqueness (that is, the number of rare alleles the candidate may possess).

Although the reduced genetic diversity among its colony animals is of great importance for increased reproducibility of vaccine development and exposure and pathogenesis experiments, colony managers at Sabana Seca Field Station might consider promoting gene flow from other primate centers to introduce more Indian-origin alleles into its conventional and SPF colonies. These strategies in turn would mitigate further genetic bottlenecks and not only abate loss of rare alleles over time due to genetic drift but also promote the introgression of novel alleles into the colony. In addition, implementing a program of systematic foster infant swaps among breeding groups can limit future differentiation among these units and effectively render the SPF breeding colony a single random-mating population.

The current study entails the first attempt to characterize the population genetics of the conventional and SPF colonies at the Sabana Seca Field Station. Our findings show that the SPF colony has several unique features including: 1) a highly homogenous and genetically stable population, reflecting slight to moderate levels of differentiation over time, 2) low estimates of inbreeding coefficient, and 3) a high degree of Indian ancestry. These population genetic characteristics support the historical origin of this population and confirm the high value of these colonies as resources for biomedical research.

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