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THE DEVELOPMENT OF A CHINESE-INDIAN HYBRID (CHINDIAN) RHESUS MACAQUE COLONY AT THE CALIFORNIA NATIONAL PRIMATE RESEARCH CENTER (CNPRC) BY INTROGRESSION

SREETHARAN KANTHASWAMY^{1,2}, LEANNE GILL², JESSICA SATKOSKI¹, VIVEK GOYAL¹, VENKAT MALLADI¹, ALEXANDER KOU¹, KIRIN BASUTA¹, LILIT SARKISYAN¹, DEBRA GEORGE¹, and DAVID GLENN SMITH^{1,2}

¹ Department of Anthropology, University of California–Davis, Davis, California

² California National Primate Research Center, University of California–Davis, Davis, California

Abstract

Fullbred Chinese and Indian rhesus macaques represent genetically distinct populations. The California National Primate Research Center introduced Chinese founders into its Indian-derived rhesus colony in response to the 1978 Indian embargo on exportation of animals for research and the concern that loss of genetic variation in the closed colony would hamper research efforts. The resulting hybrid rhesus now number well over a thousand animals and represent a growing proportion of the animals in the colony.

We characterized the population genetic structure of the hybrid colony and compared it to that of their pure Indian and Chinese progenitors. The hybrid population contains higher genetic diversity and linkage disequilibrium than their full Indian progenitors and represents a resource with unique research applications. The genetic diversity of the hybrids indicates that the strategy to introduce novel genes into the colony by hybridizing Chinese founders and their hybrid offspring with Indian-derived animals was successful.

Keywords

Microsatellites; genetic management; captive population; genetic heterogeneity; genetic structure

Introduction

In 1962 the California National Primate Research Center (CNPRC) established a rhesus macaque colony from a relatively large number of unrelated, geographically heterogeneous founder animals acquired from numerous sources throughout India. Of the approximately 4,720 rhesus macaques currently resident, these Indian-origin animals are the most frequently used research subjects at the center. The 1978 embargo by the Indian Government suspended all exportation of animals from that country and severely reduced the availability of research animals, prompting the development of domestic breeding colonies into which the introduction of new genetic material was precluded. According to the National Research Council (2003) growing demand for rhesus macaques in the United States has exceeded the domestic supply. Given the shortage, animals of Indian origin in particular are commonly re-used in multiple experimentation (Carlsson et al. 2004). The limited gene flow into the CNPRC colony was

*Correspondence Sree Kanthaswamy, PhD, Department of Anthropology, Young Hall, University of California, Davis, CA 95616, USA.

exacerbated by the subsequent development of SPF and double-derived SPF colonies as a standard in research leading to substantially smaller effective population sizes and accelerating the loss of genetic diversity.

To meet the demands for macaques for biomedical research, many domestic breeding centers that previously invested in Indian animals have resorted to purchasing animals imported from China, making that country currently the foremost purveyor of rhesus macaques for biomedical research. As a consequence, almost all rhesus macaques bred domestically for this purpose are either Indian derived, of Chinese ancestry or products of inadvertent crosses of pure Indian and Chinese animals occurring at some point along the national or international supply chain. It has been argued that the incidence of such inadvertent admixture between Indian and Chinese rhesus macaques is seriously underestimated and will significantly increase in the future (Ferguson et al. 2007) due to uncertainty of animal origins. As a result, detecting and quantifying the degree of hybridization between individuals from these genetically and geographically distinct populations and characterizing the genetic properties of hybrid populations have become areas of great interest in the field of biomedical research.

Although we know of no other US primate center that knowingly interbreeds these two regional populations, the CNPRC has been intentionally hybridizing Indian and Chinese animals since the mid-1980s to maximize the genetic heterogeneity of its colonies. Since inadvertent and unacknowledged crossing between Indian and Chinese rhesus macaques will undoubtedly increase in the future, the intentionally mixed colony at the CNPRC provides the opportunity to study the properties of the future supply of rhesus macaques available for biomedical research.

Several studies have reported genetic differences between Indian and Chinese origin rhesus macaques, including a deep divergence in their mitochondrial DNA (Kanthaswamy and Smith 2004; Smith and McDonough 2005, Satkoski et al. 2007). The genetic difference between the mitochondrial genomes of Indian and Chinese rhesus macaques (Kanthaswamy and Smith 2004; Smith and McDonough 2005) is as great as that between some congeners (e.g., that between *M. assamensis* and *M. thibetana*) regarded to belong to separate species (Hayasaka et al. 1996), and the mtDNA of Chinese rhesus macaques is more similar to that of some other macaque species (e.g., *M. cyclopis* and *M. fuscata*) than to that of Indian rhesus macaques with whom they are considered to be conspecific (Zhang and Shi 1993; Melnick et al. 1993; Morales and Melnick 1998; Tosi et al. 2003; Smith et al. 2007).

The Indian and Chinese nuclear genomes are also quite divergent, as demonstrated by their allozymes (Smith 1994), microsatellites (Morin, Kanthaswamy et al. 1997; Kanthaswamy and Smith 1998; Smith et al. 2000; Smith et al. 2006; Satkoski et al. 2007), Major Histocompatibility Complex (MHC) loci (Viray et al. 2001; Doxiadis, et al. 2003; Penedo et al. 2007) and SNPs (Ferguson et al. 2007; Hernandez et al. 2007 ; Malhi et al. 2007). Ferguson et al. (2007) and Hernandez et al. (2007) reported that between 67 and 70% of the SNPs in the rhesus genome were unique to either pure Indian or pure Chinese rhesus macaques [although this observation of population specific alleles is discordant with that of Malhi et al. (2007), perhaps resulting from the proximity of the markers chosen in the former studies to coding regions of the genome influenced by selective drive]. Satkoski et al. (2007) concluded that Chinese rhesus macaques exhibit almost twice the number of alleles per microsatellite locus and a higher level of heterozygosity than Indian animals. Opportunities for natural interbreeding between rhesus and longtail (*M. fascicularis*) macaques in the hybrid zones of Indochina might also contribute to the greater genetic variation among the Chinese rhesus macaques compared to Indian animals (Tosi et al. 2002, 2003, Kanthaswamy et al. submitted). In contrast, marked genetic homogeneity of Indian rhesus macaques probably resulted from a

genetic bottleneck/founder effect they experienced near the last Pleistocene interglacial period (Hernandez et al. 2007).

Demonstrable levels of phenotypic differences between the two regional varieties of rhesus macaques also exist, much of which undoubtedly result from genetic differences. Indian rhesus macaques have been observed to be less aggressive than the Chinese or Chinese-Indian hybrid animals (Champoux et al. 1997). Eastern and western-specific differences in blood chemistry, morphology, physiology have also been reported (Smith and Scott, 1989; Champoux 1996, 1997; Clark and O' Neal, 1999).

Of great biomedical relevance is the fact that Indian animals have often been considered a better model for HIV research than Chinese animals because Indian animals develop SIV symptoms that mirror a human's with AIDS in terms of disease patterns, incidence and severity (Cohen 2000). Chinese animals have been observed to succumb to SIV more gradually than Indian animals and thus were considered poor subjects for studies on HIV (Joag et al., 1994; Cohen, 2000; Ling et al., 2002). These reports have limited the use of rhesus macaques of Chinese origin in AIDS research. However, Burdo et al. (2005) showed that Chinese animals inoculated with SIV serially passed through Chinese rhesus macaques, instead of Indian rhesus macaques as in previous studies, experienced an SIV pathogenesis that was identical to that of Indian rhesus macaques inoculated with the same viral strain that had been repeatedly passed through rhesus macaques of Indian origin. They concluded that SIV becomes more virulent as it adapts to its host and, therefore, that the differential response to SIV infection in macaques is predominantly a vector, not a host, response.

Nevertheless, the well characterized genetic differences between Indian and Chinese rhesus macaques compel genomic studies of the new man-made hybrid populations. Genetic variation that reflects the differences in geographic origin among research subjects contributes to allele frequency heterogeneity and has implications for design of biomedical experiments. While linkage studies of families are an efficient way to map Mendelian genes, association studies can be effective for mapping complex disease genes with weak penetrance. For instance, the notable differences in temperament, blood chemistry and monoamine levels between rhesus monkey infants derived from pure Indian animals and Chinese-Indian hybrids reported by Champoux et al. (1994, 1996, 1997) provide the rationale for comparative QTL or disease mapping using pure Indian and Chinese origin animals and hybrid Indian/Chinese animals. Thus, given a disease whose incidence differs between Indian and Chinese rhesus macaques, hybrids with disease phenotypes should exhibit an increased probability of having inherited alleles derived from that origin population (Indian or Chinese) with genes conferring susceptibility or genes closely linked to those genes. This occurs because genetic markers linked to a phenotypic outcome that differ in frequency in the purebred animals will exhibit linkage disequilibrium (LD) in affected hybrid animals that have descended from recent admixture.

Hence, as the rate of inter- and intra chromosomal recombination differ between the parental and hybrid populations (Lynn et al. 2000), hybrid animals with a given characteristic common in only Indian rhesus macaques will exhibit higher frequencies of Indian-specific genetic markers near the region where the underlying genes associated with this phenotype are located. This principle forms the basis for admixture mapping, the most experimentally practical method of detecting risk factors for common diseases (Reich and Patterson 2005).

In addition, when subjects from both India and China are combined as subjects of experiments, high inter-animal additive genetic variances increase phenotypic variance in full breed animals and can obscure correlates under study (Falconer 1960; Hartl and Clark 1989). In contrast, the phenotypic variance for traits under study are expected to be lower in randomly mixed hybrids

due to increased heterozygosity and processes such as canalization, developmental stability (Reale and Roff, 2003; Pelabon et al., 2004; Santos et al., 2005) and stabilizing selection.

In theory, hybrid rhesus macaques can be identified by their possession of alleles that are absent in Indian rhesus macaques and others that are rare or absent in Chinese rhesus macaques. For example, first generation hybrids ought to exhibit alleles uniquely characteristic of both regional varieties of regional macaques (Smith et al. 2006). It is important to identify the alleles unique to pure Indian and pure Chinese animals so that the relative proportion of Indian and Chinese ancestry of animals of unknown origin or lacking pedigree records can be approximated with a high level of confidence. While appropriate genetic markers can distinguish pure Indian and Chinese rhesus macaques (Smith and McDonough, 2005; Smith, 2005; Smith et al., 2006; Satkoski et al 2007) from first generation Indian-Chinese hybrids, identifying the relative genetic contribution of each group in subsequent hybrid offspring is more challenging and requires many more polymorphic loci than are typically needed for parentage testing. This is because multiple generations of hybridization between Indian and Chinese rhesus macaques (see Table 1) produce offspring whose range of ancestry is much broader than that for first generation hybrids and, therefore, more difficult to accurately predict even with a large number of highly informative markers. Additionally, most hybrid classes can be generated in multiple ways (i.e., a $\frac{1}{4}$ Chinese animal can be generated through two different types of matings, but co-adapted gene complexes and linkage groups will be more disrupted in the half Chinese-half Chinese crosses than in the full Indian-half Chinese crosses).

In this study, genotypes for 13 autosomal microsatellite loci together with demographic records were used to determine parentage, construct pedigrees of the animals at the CNPRC and estimate proportions of Indian and Chinese ancestry in each animal. The genetic structures of different classes of Chinese-Indian hybrid or “Chindian” rhesus macaques, based on proportion of Chinese ancestry were compared both to quantify the genetic change in this population over up to five generations of admixture time and to identify the unique genetic characteristics of each hybrid class. Comparisons of full breed animals and their hybrid offspring can not only reveal the otherwise unpredictable genetic structure of the hybrid population that has emerged but also facilitate the development of the hybrid classes (e.g., half-Indian/half-Chinese) for specific types of biomedical or behavioral experimentation based on their genetic backgrounds, such as admixture mapping.

Methodology

Fragment analysis of the 13 microsatellites (Table 2) and parentage testing were performed at the Veterinary Genetics Laboratory (VGL) at the University of California, Davis, CA. Parentage verification is based on both parents' genetic profiles, one or the other parent's profiles or based on the individual animal's genotype record only. When the genetic information of the alleged dam is not on file and the sire is unknown, behavioral and circumstantial evidence, such as the presence of the purported parents in the same cage during the time of conception of an individual animal and the female with whom an individual animal as an infant secures maternal attention/affection, is used.

These markers were selected for this study on the basis of their 100% completeness of genotype data and are part of a larger panel that is routinely used for parentage testing at the VGL. Three of these markers flank the core Major Histocompatibility Complex (MHC) region on the rhesus macaque chromosome 4; D6S291 is proximal to the centromere while D6S276 and D6S1691 are positioned closer to the telomere (Penedo et al. 2005). They estimated genetic distances of 9.7 cM between D6S291 and D6S276 and 0.4 cM between the latter and D6S1691. The remaining markers lie on different chromosomes and, therefore, are not physically linked.

Over 1,300 animals including full blood Indian, full blood Chinese and hybrid individuals were involved in the establishment of the Chinese-Indian, or Chindian, rhesus macaque colony at the CNPRC. Only animals with no missing genotype data at any of the loci were included in our analyses, providing data from a total of 762 animals representing entire pedigrees (including great-great-grandparents, great-grandparents, grandparents, parents and offspring) for this study. Most first generation hybrids (half Indian-half Chinese animals) were backcrossed (i.e., bred with fullbreed animals whose ancestry matches that of one of its parents) with fullbreed Indian rhesus macaques to produce hybrids that are of $\frac{1}{4}$ Chinese ancestry. Most of these were, in turn, backcrossed with full breed Indian animals to produce hybrids of $\frac{1}{8}$ Chinese ancestry (Table 3). Therefore, most animals of $\frac{1}{2}$, $\frac{1}{4}$ and $\frac{1}{8}$ Chinese ancestry are, respectively, first, second and third generation hybrid backcrosses with fullbreed Indian rhesus macaques and reflect progressive introgression of genes of Chinese origin into the predominantly Indian rhesus colony. Hybrids of these three fractions of Chinese ancestry ($\frac{1}{8}$, $\frac{1}{4}$ and $\frac{1}{2}$) as well as all others (i.e., $\frac{3}{8}$, $\frac{5}{8}$, $\frac{3}{4}$ and $\frac{7}{8}$), appear in the third and fourth hybrid generations, although hybrids with a relatively high proportion of Chinese ancestry (e.g., $\frac{5}{8}$, $\frac{3}{4}$ and $\frac{7}{8}$) are much rarer than those with half or less Chinese ancestry. The animals were categorized into hybrid classes based on their proportion of Chinese ancestry (0 [or pure Indian animals], $\frac{1}{8}$, $\frac{1}{4}$, $\frac{3}{8}$, $\frac{1}{2}$, $\frac{5}{8}$, $\frac{3}{4}$, $\frac{7}{8}$ and 1, or full Chinese) and the generation of their cross (first through fourth) was approximated based on the demographic records at the CNPRC recording their years of birth. The $\frac{7}{8}$ Chinese hybrid animals were not included in the population genetic analysis because there were only three such animals, only two of which had complete genotype profiles for each of the 13 loci analyzed. The numbers of animals employed in this study are provided in Tables 4 and 5.

The PopGene v.1.32 computer program (Yeh and Boyle 1997) was used to estimate allele frequencies, observed heterozygosity (H_o) and gene diversity or expected heterozygosity (H_e ; see Nei, 1987) within each purebred and each hybrid/generation class (e.g., $\frac{1}{2}$ Chinese hybrids can result from either first, second, third or fourth generation crosses). The effective allele number (n_e , the number of equally frequent alleles sufficient to provide the same level of homozygosity as that actually observed; Hartl and Clark, 1989), was estimated as the reciprocal of $1 - H_e$ (Nei, 1987). The PopGene software was also used to calculate F_{is} , F_{it} and F_{st} values to examine the genetic structure this population. The GENEPOP (Rousset and Raymond 1995) program was used to determine the level of pairwise genetic differentiation among the pure bred and hybrid groups using Weir and Cockerham's pairwise F_{st} (1984).

The GENEPOP program was used to test for the presence of linkage disequilibrium (LD, or the non-random association of genotypes occurring at different loci) among the 13 loci, using the exact probability test. The null hypothesis is that genotypes at one locus segregate independently of genotypes at the other unlinked locus. Unbiased estimates by randomization (1,000 iterations) and by the Markov-chain method for the exact probabilities of random association for all contingency tables corresponding to all possible pairs of loci within each population were used to test the null hypothesis of absence of association between alleles at different loci at the 0.05 level of probability.

To further distinguish the allele frequencies representative of the two regional populations we used the program STRUCTURE (Pritchard et al. 2000) to calculate the expected allelic frequencies of individuals in each subpopulation based on an assignment index and determine the relative probabilities of assignment of an animal to each of those subpopulations based on that animal's genotypes. The analysis was run at sweeps of 10^5 iterations after a burn-in period of 10^5 with and without *a priori* population information.

Results

Based on the 13 microsatellite loci, the average observed (H_o) and expected (H_e) heterozygosity estimates for all animals tested were 0.82 and 0.83, respectively (Table 4). As previously reported (Satkoski et al. 2007), full breed Chinese rhesus exhibited higher values of H_o (0.84) and H_e (0.86), similar to those estimates for the entire sample, than full breed Indian rhesus (0.78 and 0.79, respectively). The $\frac{1}{2}$ Chinese hybrids exhibited the highest level of H_o at 0.86, while the full Chinese animals exhibited the highest level of H_e at 0.86. All hybrid classes exhibited levels of heterozygosity that were higher than the Indian full breed animals. Overall, estimates of H_e and H_o were higher in pure Chinese and hybrid animals compared to the pure Indian group alone by over 7% and 6%, respectively. When 25 animals were randomly selected from the pure Indian and each of the hybrid classes and the heterozygosity values compared, the values were not significantly different from those calculated from the entire population, demonstrating that the similarities between hybrid and pure Chinese individuals are unaffected by sample size.

The mean actual and effective allele numbers in each hybrid rhesus macaque group were also higher than those in the full breed Indian rhesus macaques (Table 4). The high effective (n_e) relative to the actual allele numbers (n_a) in the pure Chinese animals suggests that only the common alleles are being represented due to the small sample size. The cross-generational average estimates of n_a and n_e were 13.3 and 6.8, respectively. Unsurprisingly, given the higher allele numbers typically observed in Chinese rhesus (Satkoski et al. 2007), allelic numbers have steadily increased in the CNPRC Chindian population over the two decades since the first pure Chinese rhesus were introduced (Table 5). In contrast, estimates of H_o and H_e only increased in the early to mid-1980s when the Chinese origin animals were first introduced resulting in the first F1 births maximizing overall colony heterozygosity but these estimates remained constant since then because heterozygosity, as the 25-sample random tests performed here have also revealed, is less influenced by sample size than are allelic numbers.

The among-group mean F_{is} , F_{it} and F_{st} estimates for all 13 loci were -0.005 , 0.018 and 0.023 . The F_{st} estimates reflect that most of the genetic variance in the Chindian colony results from variation in the proportion of Chinese ancestry, consistent with the excess of H_e over H_o , which commonly results from population subdivision.

Pairwise F_{st} estimates ranged from -0.005 (between $\frac{3}{4}$ Chinese animals and pure Chinese animals) to 0.05 (between pure Chinese and pure Indian animals; Table 6). Despite some inconsistencies, pairwise differences among the different pure and hybrid animals generally corresponded with their recorded degree of genealogical relationship. Estimates of genetic identity according to Nei (1987) also confirmed this genetic relationship among the pure Indian, Chinese and the intermediate genealogical hybrid groups (Table 6). The pure Indian animals were more similar to the hybrid clusters, even those with $\frac{5}{8}$ Chinese ancestry, than were the Chinese origin animals. The $\frac{3}{4}$ Chinese hybrids, however, exhibited the highest genetic similarity to the pure Chinese animals, reflecting a gradual replacement of Indian alleles by Chinese introgression in the Chindian rhesus colony (Table 6).

As Table 7 indicates, the frequency of assignment of Chindian rhesus macaques to Chinese ancestry by the STRUCTURE analysis is approximately proportional to percentage of Chinese ancestry. Predictably, the half Chinese-half Indian hybrids were assigned with approximately equal frequency to the Chinese and Indian groups. The analysis was more successful in differentiating the pure Indian and $\frac{1}{8}$ Chinese animals from the other groups than distinguishing any of the other groups from each other (data not shown). With only a few exceptions, STRUCTURE generally failed to distinguish full Chinese animals from partially Chinese animals. This probably stems from the marked genetic homogeneity of pure Indian

rhesus macaques compared with pure Chinese and hybrid animals. As with the heterozygosity data, any amount of Chinese genetic introgression appeared sufficient to mute the Indian genetic contribution.

Statistical tests for linkage disequilibrium (LD) between pairs of the 13 microsatellite loci showed that 6.5% of the pairwise tests had significant results ($P < 0.05$) in the pure Indian animals (Table 8). No data were obtained from the fullbreed Chinese samples due to the small number of animals ($N = 9$) employed in this study. Between 0% and 31% of the possible tests among the hybrid animals showed significance at the 0.05 level of probability. The 1/8, 1/4 and 1/2 hybrids animals, whose sample sizes are largest, all showed a higher degree of pairwise associations among the alleles at different loci than the pure Indian animals. The 5/8 Chinese animals were discrepant in exhibiting no associations among their loci, but their sample size ($N = 18$) was probably too low to detect a statistically significant association between loci with such large numbers of alleles. As expected, admixture maximizes linkage disequilibrium among the first generation hybrids, i.e., the 1/2 Chinese animals, which exhibited the highest pairwise associations (31%) between loci and dissipates (albeit irregularly) with increased recombination. Interestingly, statistical tests for LD among physically linked markers D6S291, D6S276 and D6S1691 revealed no significant results ($P < 0.05$) in any of the pure bred and hybrid animal categories.

Discussion

Before the genetic management of captive colonies became widely accepted as a valuable tool in most US primate centers, the CNPRC used marker-based parentage assessment to construct pedigrees, introduced founders from diverse geographic origins and promoted intra-colony gene flow to curb genetic subdivisions and inbreeding with the colony to enhance the production of genetically characterized stocks for research, with the additional benefit of conserving the colony's genetic diversity (Kanthaswamy et al. 2002, 2006). With the reduced availability and increased price of rhesus macaques, especially highly derived SPF animals, genetic management of existing animals proved to be essential to ensure that high quality research animals are available in the long-term.

The CNPRC has always fostered gene flow to its colony by importing a few hundred animals over the years from different geographic regions for use in the colony's breeding program. Since the 1980s the CNPRC has consistently used Chinese founders to introduce new alleles into the predominantly Indian colony. This hybrid colony originated with a breeding group consisting of 40 subadult pure Indian females, seven juvenile/subadult pure Indian males and three adult pure Chinese males. Since 1983 (Smith 1986), the hybrid offspring of the pure Chinese sires and pure Indian dams (with 1/2 Chinese ancestry) and those of hybrid females and the full breed Indian sires when they reached sexual maturity (with 1/4 Chinese ancestry) were employed in foster infant exchanges with other Indian rhesus macaque breeding groups and in the formation of new predominantly Indian groups at CNPRC, for the purpose of reducing genetic differentiation (genetic subdivision) among different captive groups. Due to this intentional distribution of hybrids, Chindian rhesus macaques represent a growing proportion of animals in the colony.

Due to the breeding patterns described above, the majority of Chindian animals, even those few with predominantly Chinese ancestry, exhibit mtDNA characteristic of Indian macaques. Segregating nuclear markers do not reflect this female-biased introgression and can be uniquely characteristic of either Indian or Chinese ancestry based on the proportion of an individual's genome originating from each set of subpopulations (Avice 1994; Ferguson et al. 2007; Satkoski et al. 2007). Although we could not differentiate hybrid classes based purely on these genetic markers, a far greater number of sufficiently informative nuclear alleles could be

identified and used not only to distinguish hybrids and purebred individuals but also to identify the proportion of Chinese ancestry of hybrids (Anderson and Thompson 2001, Smith et al. 2006, Satkoski et al. 2007).

Kanthaswamy and Smith (1998), Morin et al. (1997), Penedo et al. (2007), and Satkoski et al. (2007) have all reported that Indian-origin animals are consistently more genetically homogenous than the Chinese animals, probably due to a genetic bottleneck in the Indian rhesus macaque population (Hernandez et al. 2007). This genetic homogeneity has probably contributed to the desirability of the Indian variety in biomedical research (Smith et al. 2006). Due to the high LD [most likely imposed by the past bottleneck (Hernandez et al. 2007) and/or some gene flow from Burma (Smith and McDonough 2005, Kanthaswamy et al. in submitted)] in Indian rhesus macaques, far fewer loci would be required when Indian rhesus macaques are used for whole genome association studies, relative to Chinese macaques.

Smith and Scott (1989) argued that under the prevailing ban on Indian animals, the supply of domestic Indian-derived animals will become even more genetically homogeneous. Therefore, interbreeding Chinese and Indian origin animals was proposed to foster increasing diversity among colony animals. Our results show that the introduction of Chinese animals into the predominantly Indian colony has in fact increased the level of genetic diversity among the genetically mixed offspring generations to a level approximating that in full blood Chinese rhesus macaques despite a relatively low level of Chinese admixture. The level of heterozygosity, greatest among F1 hybrid offspring, was actually more similar to pure Chinese rhesus macaques – this similarity extended to animals with 1/8 Chinese ancestry in some of the F3 hybrid offspring.

The introduction of novel genes of Chinese origin into the CNPRC colony of primarily Indian-derived animals produced a sharp increase in both allele number and heterozygosity, producing an overall more genetically diverse animal. Although crossbreeding these two regional populations will maximize heterozygosity, it also has the potential to increase the expression of heterosis (Falconer 1960), which acts reciprocally to the effects of inbreeding and LD (Smith and Short 1989, Falconer and Mackay 1996). Champoux et al.'s (1994, 1997) observation of increased aggressive behavior among Chindian hybrids than among pure Indian parents suggests that heterosis for emotional reactivity is probably evident in the Chindian hybrids implying that these hybrid animals and pure bred animals might be appropriate models for studying genetics of neuropsychiatric-related outcomes and better understanding the molecular mechanism of heterosis in general.

The extensive LD among physically unlinked loci in the 1/8, 1/4 and 1/2 Chinese hybrid rhesus macaques resulted from admixing regional populations with very divergent genotype frequencies. The high LD scores persisting among linked loci in Indian rhesus macaques reported by Hernandez et al. (2007) probably resulted from severe genetic bottlenecks/founder effects during the previous interglacial period that reduced genetic heterogeneity as well as subsequent hybridization with Burmese rhesus macaques to their southeast (Smith and McDonough, 2005). Such evolutionary forces can modify gene pools rendering some populations more suitable for studies of QTLs and disease association. Conversely, past or ongoing natural population expansions among wild populations and widespread human mediated admixture as has apparently occurred among captive bred rhesus macaques bred in China (Satkoski et al. 2007) retard the decay of LD (Laan and Paabo 1997 and 1998, Terwilliger et al. 1998).

In contrast, LD among unlinked loci in full breed Indian rhesus macaques at the CNPRC has decayed significantly probably reflecting decades of colony management's efforts to retain or maximize the colony's genetic diversity and minimize genetic subdivision among breeding

groups in the colony. The transient increase in LD among unlinked loci in the 1/8, 1/4 and 1/2 Chinese animals renders them ideal subjects for QTL and disease association studies. If a similar level of LD is observed among syntenic loci, subsequent generations of hybrid animals that experience LD decay at a faster rate may provide better opportunities for locating potential candidate genes that are difficult to localize in the genome as a result of extensive LD among candidate genes in the preceding generations of hybrids. Thus, our analysis of the genetic makeup of pure Indian, Chinese and Chindian animals at the CNPRC will be important for LD-based disease gene localization.

Information from genetic relationships among regionally representative rhesus macaques and different hybrid classes like that presented in this study, as well as genetic information based on a larger number of informative markers (such as SNPs) could provide critical information relating to several aspects of comparative biomedical research. Since substantial advantage may be gained through access to multiple populations with divergent population histories, coupled with complete colony demographics and captive management records, this information when used in a comprehensive manner could significantly benefit primate research.

As a precaution, rather than conducting hybrid backcrosses to full blood Chinese rhesus macaques exclusively and enriching Chinese genes into the colony, the CNPRC ought to continue to backcross their Chindian animals with the pure Indian-derived ones so that the alleles unique to the Indian animals will be retained in the domestic gene pool. This approach will help ensure that alleles that are important to the co-adapted gene complexes such as the MHC will not be steadily eliminated from our captive colonies and that maximum allele diversity will be retained. In this manner, a near full blood Indian colony with high heterozygosity, high allele numbers, lower genetic variance and stronger LD than either purebred Indian or Chinese rhesus macaques can be produced.

Given the degree of genetic admixture, the Chindian rhesus macaques at the CNPRC may be suboptimal research models in experimental protocols where pure Indian and Chinese animals are deemed most suitable. On the other hand, this man-made hybrid population may respond very differently to other types of experimentation involving traits which have a phenotypic variance that is lower than that between Indian and Chinese rhesus macaques making the hybrids a new biomedical resource. Except for reports on temperament and aggressive behavior by Champoux et al. (1994, 1997), who showed that Indian-derived infants expressed increasingly different behavioral patterns than hybrid infants over a four week-long procedure of repetitive social-separation and reunion, few studies have sought to understand the suitability and use of hybrid rhesus macaques for specific research on phenotypes and/or develop them as models for those experimental programs although it would be interesting to know at which hybrid class the behavioral differences would dissipate.

Burdo et al.'s (2005) study that shows that SIV viral set-points in rhesus macaques are not restricted to the region of these animals' geographic origin or ancestry substantiates the belief that within the context of the SIV/rhesus model both the Chinese rhesus macaques and the Chindian hybrid classes may indeed be well-suited as alternatives to or replacements of pure Indian macaques in HIV/AIDS research. As Chinese and Indian rhesus macaques share a high degree of genetic similarity, it would be interesting to establish a basis for the disease differences at the molecular level. Greater research focus is needed not only to understand why Chinese or Indian rhesus macaques are optimal models for some experiments but also why they are suboptimal models for others.

Perhaps the Chindian hybrids will prove to be good research subjects to tease out the underlying molecular differences that govern different SIV-related immunogenetic outcomes in Chinese

and Indian origin animals. Because Chindian animals that belong to different hybrid classes should experience varying probabilities of inheriting DNA from the parental population uniquely or predominantly exhibiting a particular trait or disease-causing gene, researchers will be better able to select a particular class of hybrids as models for a specific experiment based on *a priori* knowledge. For instance, by comparing gene differential expression in SIV viral loads among the 1/8, 1/4 and 1/2 Chindians, which display extensive LD, and pure Indian and Chinese animals with widespread LD decay, the virulence of the virus in Indian, Chinese or Chindian rhesus macaques inoculated with viral strains reciprocally passing through one or the other taxon may be better understood.

The overall increase in the genetic heterogeneity of the hybrid colony at the CNPRC vindicates the decision by colony managers to introgress novel Chinese genes into the colony gene pool by hybridizing Chinese founders and their hybrid offspring with Indian-derived animals. Primate breeding facilities experiencing shortages of Indian rhesus macaques should consider interbreeding their Indian-derived animals with Chinese origin animals for maximizing colony genetic diversity, introducing novel genes into their colony and restoring LD. However, to attain a successful crossbreeding program colony managers have to know the country of origin of the founder animals and establish multi-generational pedigrees based on routine parentage testing. The more genetic information colony managers have on their captive animals, the better researchers are able to identify traits of interest and crucial host-related variables.

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Table 1

Possible matings among pure Indian, pure and Chinese and hybrid animals that produce the various intermediate genealogical classes at the CNPRC.

	Full Chinese	7/8 Chinese	3/4 Chinese	5/8 Chinese	1/2 Chinese	3/8 Chinese	1/4 Chinese	1/8 Chinese	Full Indian
Full Chinese	Full Chinese		7/8 Chinese		3/4 Chinese		5/8 Chinese		1/2 Chinese
7/8 Chinese		7/8 Chinese		3/4 Chinese		5/8 Chinese		1/2 Chinese	
3/4 Chinese			3/4 Chinese		5/8 Chinese		1/2 Chinese		7/8 Chinese
5/8 Chinese				5/8 Chinese		1/2 Chinese		3/8 Chinese	
1/2 Chinese					1/2 Chinese		3/8 Chinese		1/4 Chinese
3/8 Chinese						3/8 Chinese		1/4 Chinese	
1/4 Chinese							1/4 Chinese		1/8 Chinese
1/8 Chinese								1/8 Chinese	
Full Indian									Full Indian

Table 2

The 13 microsatellites used in this study.

Locus	Rhesus chromosomal location [*]	Repeat motif ^{**}
D3S1768	2	Tetranucleotide
D6S276	4	Dinucleotide
D6S291	4	Dinucleotide
D6S1691	4	Dinucleotide
D7S513	3	Dinucleotide
D8S1106	8	Tetranucleotide
D10S1412	9	Trinucleotide
D11S925	14	Dinucleotide
D13S765	17	Tetranucleotide
D16S403	20	Dinucleotide
D18S72	2	Dinucleotide
MFGT21	Uncertain	Dinucleotide
MFGT22	Uncertain	Dinucleotide

^{*} Chromosomal locations of the markers are based on the NCBI Map Viewer (www.ncbi.nlm.nih.gov/mapview/) and the Southwest Foundation Rhesus Genome Map (www.snprc.org/linkage/index.html).

^{**} Based on allele sizes from genotype data

Table 3 Production of hybrid animals at the CNPRC over two decades (data includes 762 animals with complete genotype information only). Shaded boxes indicate the year an animal was acquired or a particular hybrid class was produced in the colony. N is number of animals.

1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000
2			1		1	3	1	1	1	8	3	1	1							
											6	4	17	11	24	30	23	36	38	47
							5	6	8	3	13	17	12	12	27	28	16	21	27	18
											2	2	1	3	7	10	7	12	17	8
				3		2	3	10	15	11	11	12	7	10	5	11	10	11	11	8
															1	1	1	1	8	6
											3	5	5	12	5	4	2	2	3	10
						2	6								1					
2	0	0	1	3	1	7	15	17	24	22	38	41	43	48	70	84	59	83	104	97

Table 4

Estimates of mean actual and effective allele numbers and mean observed (H_o) and expected heterozygosity (H_e) across all loci. N is number of animals.

	N = 762	n_a	n_e	H_e	H_o
Full Indian	26	9.5	5.2	0.79	0.78
1/8 Chinese	236	16.6	7.1	0.83	0.82
1/4 Chinese	213	16.3	6.9	0.83	0.83
3/8 Chinese	69	14.4	7.1	0.84	0.84
1/2 Chinese	139	16.1	7.6	0.85	0.86
5/8 Chinese	18	10.6	6	0.83	0.82
3/4 Chinese	52	14.2	7.6	0.85	0.83
Full Chinese	9	9	6.6	0.86	0.84
		Mean = 13.3	Mean = 6.8	Mean = 0.83	Mean = 0.82

Table 5

Changes in H_o and H_e estimates over 20 years in five year intervals. N is number of animals.

Year	Animal ID Prefix*	N = 762	n_a	n_e	H_e	H_o
>1965–80	7059, 16–19**	5	4.3	3.3	0.72	0.72
1981–85	20–24	27	12.9	7.0	0.85	0.84
1986–90	25–29	142	16.3	7.0	0.84	0.84
1991–95	30–34	304	16.9	7.1	0.84	0.82
1996–2000	35–37	284	17.2	7.1	0.83	0.84
			Mean = 13.5	Mean = 6.3	Mean = 0.82	Mean = 0.81

* animals born in same year have same prefix and prefix increases by 1 each year

** these five individuals are all fullbreed Indian animals

Table 6

Estimates of genetic identity (above diagonal) and pairwise F_{st} (below diagonal) among the different hybrid classes of animals. Estimates include the three MHC linked loci.

	Full Indian	1/8 Chinese	1/4 Chinese	3/8 Chinese	1/2 Chinese	5/8 Chinese	3/4 Chinese	Full Chinese
Full Indian		0.934	0.966	0.927	0.924	0.929	0.854	0.729
1/8 Chinese	0.012		0.987	0.977	0.973	0.967	0.925	0.819
1/4 Chinese	0.004	0.001		0.981	0.976	0.965	0.922	0.817
3/8 Chinese	0.012	0.002	0.001		0.975	0.944	0.941	0.868
1/2 Chinese	0.014	0.004	0.003	0.002		0.931	0.969	0.865
5/8 Chinese	0.007	-0.003	-0.001	0.003	0.007		0.852	0.728
3/4 Chinese	0.028	0.011	0.012	0.007	0.002	0.020		0.931
Full Chinese	0.046	0.021	0.022	0.010	0.011	0.034	-0.005	

Table 7

Proportion of genetic affiliation of each pre-defined populations into the Chinese and Indian clusters by STRUCTURE based on genotypes for 13 microsatellites assayed. Only animals with complete profiles were used in this analysis. N is number of animals.

Group affiliation based on breeding records	Inferred percentage of genetic affiliation based on 13 STR loci	
	Chinese	Indian
Hybrid Classes (N)		
Full Indian (26)	18	82
1/8 Chinese (236)	37	63
1/4 Chinese (213)	30	70
3/8 Chinese (69)	40	60
1/2 Chinese (139)	49	51
5/8 Chinese (18)	33	67
3/4 Chinese (52)	62	38
Full Chinese (9)	87	13

Table 8

Proportion of gametic disequilibrium among the 13 microsatellite loci including the 3 MHC-linked loci. Estimates do not include the nine full Chinese animals sampled in this study.

	Percentage of loci significant at 0.05
Full Indian	6.49
1/8 Chinese	28.57
1/4 Chinese	25.97
3/8 Chinese	6.49
1/2 Chinese	31.17
5/8 Chinese	0
3/4 Chinese	11.69