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Evaluating the genetic status of a closed colony of titi monkeys (*Callicebus cupreus*) using multigenerational pedigrees

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Abstract

Background—Pedigree metrics are essential for investigating colony genetic structure.

Methods—The genetic structure of a closed *Callicebus cupreus* colony was examined using multigenerational pedigrees.

Results—Inbreeding was low but genetic drift caused the loss of founder genome representation.

Conclusions—Pedigrees can be used to detect founder representation and prevent bottlenecks and allele loss.

Keywords

Family studies; population genetic structure; nonhuman primates; genetic management

Introduction

The California National Primate Research Center (CNPRC) houses a small closed colony of coppery titi monkeys (*Callicebus cupreus*) for multiple types of behavioral and neurobiological studies [8, 11]. According to Kaemmerer and Stevens [10], the CNPRC *C. cupreus* colony was established in 1972 using animals acquired from the Delta Regional Primate Center (Covington, Louisiana, USA). In 1978, a cohort of 29 animals from the University of Göttingen in West Germany, which had also originated from the Delta Regional Primate Center, was introduced into this colony. In 1990, 11 wild-caught titi monkeys were imported into the CNPRC colony from Leticia, Colombia; there have been no animals introduced into the colony since 1990 [7].

The *C. cupreus* are maintained as pairs of monogamous adults with one to four offspring, which upon reaching sexual maturity are separated from their parents. The ample data on the

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social structure and behavioral characteristics of *C. cupreus* [5, 15], such as pairing naive animals with those with previous parental and/or alloparental experience, as well as animal temperament and social behavior, is used to assist routine colony management. However, the colony's genetic structure and composition is not as well characterized and therefore this data is not rigorously used. A more complete understanding of the colony's genetic structure is important for the populations' long-term viability and productivity [14]. Moreover, as differences in the genetic composition of captive bred animals influence their suitability in research, knowledge of the *C. cupreus* colony genetic structure will support the development of this species as an NHP model for research [1, 9].

In 2014, an initiative was implemented to characterize the genetic structure and composition of the CNPRC *C. cupreus* colony using a panel of short tandem repeats (STRs) [14]. However, STR testing was discontinued because parentage testing was deemed unnecessary as these animals are maintained in monogamous, paired housing configurations. Moreover, there was a lack of funds for continuing the DNA-based genetic testing. As such, as a no-cost effective alternative, pedigree studies were initiated for monitoring the genetic status and structure of the *C. cupreus* colony. The CNPRC *C. cupreus* colony records on each animal's parentage and sex, as well as dates of birth and death provided the basis for establishing mutigenerational pedigrees for calculating estimates of mean kinship ($\bar{k}$), kinship value ($\overline{k_v}$), gene value ($\overline{G_v}$), genome uniqueness ($\overline{G_u}$), founder equivalents ($\overline{f_e}$) and founder genome equivalents ($\overline{f_g}$), these are population-to-individual level parameters which are now being used to facilitated long-term genetic management decisions of the colony.

Materials and Methods

This study evaluated the genetic structure of the *C. cupreus* colony by using the program PEDSYS [3] to construct and analyze the pedigrees of 49 males and 45 females. Each individual's genealogical and demographic data was used to calculate inbreeding (F) and kinship coefficients (k) [6] using PEDSYS (see Supplementary file containing sample data). F is the probability that an individual inherited identical homologous alleles from a common ancestor [16] and k is an individual's average kinship with other individuals living in the colony and reflects the expected proportion of genomic similarity stemming from identity by descent (IBD) [2]. Colony-wide k values were used to generate the average estimates of mean kinship ($\bar{k}$) and kinship value ($\overline{k_v}$) Lacy's [11] methods featured in the program PedScope [Tenset Technologies Ltd, UK] was used to generate colony-wide estimates of gene value ($\overline{G_v}$), genome uniqueness ($\overline{G_u}$), founder equivalents ($\overline{f_e}$) and founder genome equivalents ($\overline{f_g}$). The mean kinship ($\bar{k}$) of the entire colony is the average of k values among living animals in the colony. The individual kinship value (k_v) of an individual is a variant of k as the value is age-specific and is defined as the individual's expected future lifetime reproduction. Mean kinship value ($\overline{k_v}$) is the average of all living individuals' k_v values, and $\overline{k_v}$ accounts for each of their age and reproductive values or genomic contributions to future generations. $\overline{G_v}$ which is $1 - \overline{k_v}$ is used for measuring the genetic diversity of the entire colony. The G_u of an individual, which is the likelihood that the individual carries unique founder alleles, was computed using MacCluer et al.'s [12] and Lacy's [11] simulation

techniques featured in PedScope. $\overline{GU}$ is the average probability across all individuals in the colony that may have acquired unique founder alleles. The values of $\overline{f_e}$ and $\overline{f_g}$ reflect the average number of equally contributing founders which would be expected to cause the present level of genetic diversity. $\overline{f_e}$ is calculated based on the assumption that no effects from genetic drift have influenced colony-wide allele frequency while the computation of $\overline{f_g}$ assumes otherwise.

Results

The reconstructed pedigrees reveal that the maximum lifespans of *C. cupreus* males and females are 29 and 22 years, respectively. The age of study animals varied from six months to 22 years old representing approximately 11 generations. The current number of adult pairs in the breeding colony is 38, with 31 that are currently or potentially reproductive and 7 that have been removed from breeding via vasectomy or tubal ligation. The potentially reproductive pairs have from zero to 7 offspring which survived at least a week, with an average number of offspring per pair of 1.19. The formerly reproductive pairs have had from zero to 13 offspring with an average of 2.3 offspring per pair.

Inbreeding coefficients (F) ranged from 0.6 to 28.1%, and averaged at 5.8%. Over 95% of the animals surveyed exhibited an F value of less than 10%; only three animals had F values that exceeded this threshold. Analysis of genomic similarity revealed that $\bar{k}$ and $\overline{k_v}$ values were 9.6% and 9.9%, respectively, indicating low levels of genomic similarity within the study cohort. Estimates of expected effective number of founders ($\overline{f_e}=12.14$ and $\overline{f_g}=7.82$) suggest that it would have taken between eight and 12 equally contributing founders to produce the current level of genetic diversity ($\overline{GV}$ of 90.1%). The discrepancy between $\overline{f_g}$ and $\overline{f_e}$ points to the loss of unique founder alleles due to genetic drift, resulting in a $\overline{GU}$ of 1.1%.

Discussion

Due to its small population size, the *C. cupreus* colony, which has been closed since 1990 for at least 11 generations, is prone to risks of reduced genetic variation and increased inbreeding. As reflected by the $\overline{GV}$ value, an absence of gene flow because of the colony's genetic isolation is impacting its genetic diversity. As consanguineous matings influence the level of inbreeding expected in any resulting offspring, such matings have a direct impact on the retention of genetic diversity in the entire colony. Therefore, the design of new breeding pairs in this closed colony has been focused on mitigating mating among close relatives. The low colony-wide inbreeding and kinship values suggest that colony management strategies have been successful in reducing consanguinity by preventing pairing first degree relatives (i.e., mating between an offspring and its parent or full sib) and second degree relatives (i.e., mating between an individual and its grandparent, aunt/uncle or half-sibling) as well as minimizing opportunities for matings between third degree relatives (i.e., mating between an individual and its first-cousin or a great-grandparent). This selective breeding scheme has likely also resulted in estimates of negative FIS, an indication of the absence of inbreeding based on *Callicebus*-specific DNA markers [14]. As comparisons of genetic diversity and

inbreeding estimates prior to and after the viral epizootic disease that struck the CNPRC in May 2009, which killed 19 of the 65 colony animals [3], showed no loss of variability occurred among the surviving colony animals [14], the same breeding program to prevent increasing levels of inbreeding is still currently employed to manage the CNPRC *C. cupreus* colony.

Founder metrics in the present study however, indicate that the effects of genetic drift have not been effectively mitigated, resulting in the loss of some founder alleles. The lack of DNA samples from older animals, including colony founders, in Mendoza et al.'s study [14] prevented the assessment effects of genetic drift in this colony and its impact on founder alleles. Going forward, colony managers should utilize pedigree metrics to rank breeding animals according to their *GU* values to increase founder genome representation and curb future genetic bottlenecks and allele loss. As the *C. cupreus* are maintained as pairs of monogamous adults, male to female ratio in the breeding stock is balanced to maximize the effective population size (N_e) of the colony relative to its census size. However, in order to further increase genetic diversity and decrease the effects of genetic drift over time, it will require the colony managers to increase the breeding group size by introducing new animals into this colony.

This study demonstrates that multigenerational pedigrees can advance *C. cupreus* colony management strategies by facilitating the estimation of founder metrics and various other associated measures of genetic diversity. Extended pedigrees can aid in the design of safeguards against the loss of captive colony genetic diversity and value due to genetic drift and inbreeding.

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