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Articles

Primate Sanctuaries, Taxonomy and Survival: a Case Study from South Africa

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The relationship between humans and non-human primates in South Africa is problematic. On the one hand, vervet monkeys were formerly designated vermin species and could be destroyed at will. On the other hand, many people keep young vervets as pets even though this is illegal, and the animals are confiscated if discovered. Sanctuaries were established to accommodate large numbers of orphaned and confiscated animals. Owners of some of these sanctuaries attempt to establish normal troop structures in the hopes of releasing these animals back into the wild and relieving overcrowding. However, local farmers, fearing crop damage, resist this release. Nature conservation authorities also resist release fearing possible disruption of natural patterns of genetic variability even though there is no consensus on the number of subspecies or evolutionary significant units among South African vervets. We have designed a sampling strategy to aid in resolving some of the taxonomic issues preventing release. Data from microsatellite loci suggest no genetic structuring linked to geographic distribution. Coefficients of population differentiation (AMOVA) show that 96.72% of variation within South Africa occurs within populations. Addition of a reference group from Kenya in East Africa still yielded a within population value of 90.20%, suggesting limited differences between populations. This information can contribute to informed management decisions, since there is no evidence from the populations sampled to date to support the hypothesis of genetic structuring within the overall South African vervet monkey population. There is therefore no genetic support for the current restrictions on the mixing of animals at sanctuaries or releases into the wild.

KEYWORDS: vervet monkey, sanctuary, evolutionary significant unit, microsatell

Introduction

Vervet monkeys (*Chlorocebus aethiops*) are among the most widely distributed primates in the world. They are able to live in a variety of habitats. This broad adaptability brings them into conflict with humans, as they will frequently use cultivated products to supplement natural forage. As a result, farmers and gardeners regard vervets as problem animals. Vervet monkeys, in addition to baboons, caracal and jackal species, were formerly subjected to the recently repealed South African Problem Animal Control Ordinance ("Ordinance 26, 1957") which allowed them to be destroyed as pests. On the other hand, young vervet monkeys are often kept as pets by South African families. This practice is illegal and usually ends with the monkeys being confiscated by conservation authorities. This duality – pest and pet – led to a situation where orphaned and confiscated animals in great numbers were placed in rehabilitation facilities throughout South Africa. The goal of these rehabilitation centers is to try to reintroduce animals into the wild. These centers are currently overcrowded and want to release animals.

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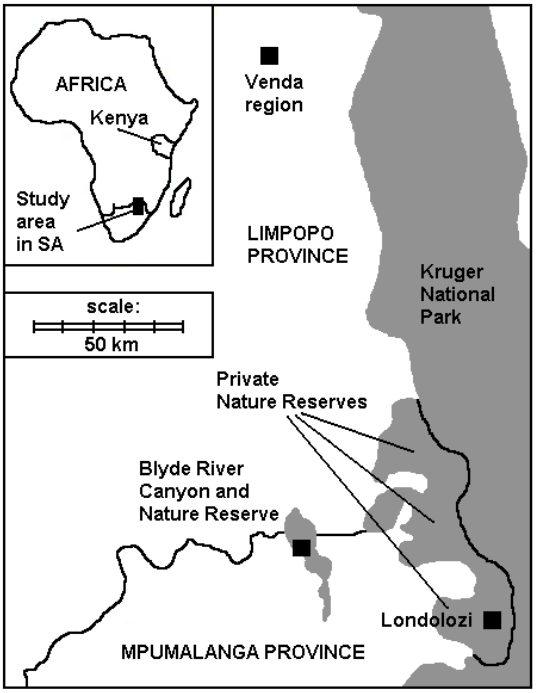
The South African conservation authorities have expressed concern that there may be genetic structuring in the southern African vervet monkey populations, in line with the Evolutionary Significant Unit (ESU) concept (Moritz 1994, 2002; Waples 1995). Forming troops of rehabilitated monkeys at sanctuaries ignores possible genetic structuring, since animals are often placed together without regard to provenance. Releases of rehabilitated troops back into the wild could therefore result in the disruption of natural patterns of genetic diversity. For this reason, conservation authorities have imposed stringent regulations for sanctuaries. The regulations entail microchip marking of animals, separate cages for animals originating from different areas, and a general ban on releases back into the wild.

The current situation is untenable, since there are presently approximately 3,000 vervet monkeys at sanctuaries in South Africa. The situation can be resolved thorough molecular study of the animals. There has been considerable debate on the units, terminology and criteria for conservation of geographic genetic variants with varying levels of evolutionary potential (Bowen 1998). We believe that application of the ESU concept will be useful in resolving this issue. Moritz (2002) set specific criteria for recognition of ESUs, based on reciprocal monophyly for mtDNA markers and significant allele frequency differences for nuclear markers. To genetic considerations, Waples (1995) added the importance of local populations to the ecological and genetic diversity of the species. Vogler and DeSalle (1994) suggested that a biological unit is an ESU only if all individuals in the unit share at least one heritable trait never found in any individuals from any other units.

There have been previous studies of genetic structuring of vervet monkeys in Ethiopia and Kenya using both electrophoretic (Turner 1981; Dracopoli et al. 1983) and nuclear polymorphisms (Turner et al., 2000). A previous analysis of genetic structuring in vervet monkeys in South Africa was published by Grobler and Matlala (2002). This allozyme-based study reported genetic structuring based on one diagnostic locus (*Prt-2*), with private alleles in two out of three regional populations screened and with an overall F_{ST} value (between regional populations) of 0.046. These authors recommended that mixing and releases be discouraged pending the results of more elaborate genetic screening. The aim of the present study was to gauge the extent of genetic structuring in the overall South African vervet monkey population, using appropriate modern molecular techniques for genetic analysis.



Methods

Sample sites and collection

Vervet monkeys (n=36) were sampled from four localities in South Africa (Fig. 1): the Blyde River Nature Reserve (nine animals sampled from one troop), Londolozi Private Nature Reserve (10 and 14 animals respectively, sampled from two troops) and the Venda region (three animals sampled from one troop). These reserves host vervet monkeys that occur naturally within the distribution range of the species. The three localities are isolated by distance as well as environmental conditions, which should introduce a component of adaptive significance (if present) to pure geographical distance. Altitude and rainfall figures for the three localities are as follows: Blyde River: 1,600m / 3,000mm; Londolozi: 800m / 500mm; and Venda: 697m / 700mm. Animals were collected using drop-traps and sedated using Zolotil. Ear clippings, blood samples and hair samples were taken for genetic analysis. Vervet monkeys from Kenya (207 animals from eight troops) were included as an outgroup.

Genetic analysis

Genetic screening was based on microsatellite markers. We used the loci D1S518, D5S1466, D11S956 and D15S108. Microsatellite fragments were amplified in 7 µl PCR reaction volumes, with the forward primers labeled with fluorescent dyes. The reaction mixture consisted of 25-50ng DNA, 4pmol of each primer, 0.5 U DNA polymerase, 1X buffer, 0.25mM dNTP mixture, and 1.5mM MgCl2. Reaction conditions were 10 min at 95°C, followed by 35 cycles each of: 45 s at 95°C, 80 s at 58°C and 80 s at 72°C, and with a final extension step of 10 min at 72°C. Analysis of microsatellite fragments was performed on an ABI377 automated sequencer. GENESCAN© and GENOTYPER© software were used for initial scoring of fragments.

Statistical analysis

Analyses of microsatellite data started with testing for linkage disequilibrium (Weir 1979), using POPGENE (Yeh and Yang 1999) software. We also used this software to calculate average allelic frequencies and the significance of allelic frequency differences among regional populations (using a chi-square test). To compare variation within and between populations, we performed an analysis of molecular variation (AMOVA) as described by Michalakis and Excoffier (1996) and implemented in ARLEQUIN (Schneider et al. 2000). Levels of differentiation between populations were estimated using R_{ST} (Slatkin 1995), a coefficient based on the stepwise mutation model, and using RST CALC (Goodman 1997). To give scale to the values obtained for the AMOVA and R_{ST} coefficients, calculations were repeated using the vervet monkeys from Kenya as outgroup.

Results

Results from microsatellies showed no influence of linkage disequilibrium among the four loci used, and all loci were thus usable for further statistical analyses. There were no fixed allelic differences among populations from South Africa. Significance of allele frequency differences among the three regional groups are presented in Table 1. Only one pair of allele frequencies (from 12 compared) differed significantly (P=0.01, for D15S108 between Londolozi and Blyde River). Values from AMOVA showed that only 1.11% of total variation occurred among the three regional South African populations. Between troop variation (from the two troops at Londolozi) accounted for 2.17% of variation, with the remaining 96.72% of variation found within troops. R_{ST} values (and gene flow) between pairwise combinations of the four populations screened are shown in Table 2. None of the values suggested significant (P=0.05) differentiation. Addition of the vervet monkey data from Kenya resulted in a slight increase in the among region component of total variation, at 7.77%. The among group component was 2.04%, with 90.20% of total variation found within troops. R_{ST} values did indicate significant (P=0.01) structuring between the South African and Kenyan populations (Table 2).

	D1S518	D5S1466	D11S956	D15S108
Blyde – Londolozi	0.29	0.05*	0.66	0.01*
Blyde – Venda	0.89	0.26	0.53	0.28
Londolozi – Venda	0.39	0.64	0.61	0.28

Table 1. Significance of allele frequency differences between South African regional populations. Significant (P<0.05) differences between allelic frequencies scored in population pairs are indicated with *.

	Londolozi(1)	Londolozi(2)	Blyde	Venda
Londolozi(2)	R_{ST}=0.09	-	-	-
	P=0.12	-	-	-
	Nm=2.64	-	-	-
Blyde	R_{ST}=0.0	R_{ST}=0.17	-	-
	P=0.41	P=0.06	-	-
	Nm=infinite	Nm=1.20	-	-
Venda	R_{ST}=0.11	R_{ST}=0.00	R_{ST}=0.15	-
	P=0.29	P=0.56	P=0.13	-
	Nm=2.07	Nm=infinite	Nm=1.42	-
Kenya	R_{ST}=0.68	R_{ST}=0.69	R_{ST}=0.62	R_{ST}=0.70
	P=0.01*	P=0.01*	P=0.01*	P=0.01*
	Nm=0.12	Nm=0.11	Nm=0.15	Nm=0.11

Table 2. Differentiation (RST) and gene flow (Nm) among vervet monkey populations from three regions in South Africa, and among vervet monkey populations from South Africa and Kenya. P values marked with * denote significant differentiation.

Discussion

Based on the limited data currently available, vervet monkeys as a group appear to be relatively homogenous. Results from AMOVA suggested that only 1.1% of variation occurs between regional groups. Comparative microsatellite-based data for African primates is not available. However, for other African mammals, it is notable that a between regional population AMOVA value of 12.8% was reported by Grobler et al. (2005) for nyala (*Tragelaphus angasii*). Considering that this value refers to an antelope with presumably much higher mobility compare to vervets, the value of 1.1% obtained for vervet monkeys suggest an extremely low level of genetic structuring within the species. This trend is supported by the fact that no R_{ST} values suggested significant differentiation between pairwise combinations of populations. Finally, only one out of 12 pairwise comparisons of allelic frequencies suggested a significant difference, providing very limited support for the criterion of Moritz (2002) to recognize ESUs within species.

There is thus in this limited sample no evidence to date to support the hypothesis of genetic structuring within the overall South African vervet monkey population, and therefore no genetic support for the current restrictions on the mixing of animals at sanctuaries prior to releases into the wild. Nevertheless, a final recommendation on translocations of vervet monkeys can only be done following a more elaborate screening of genetic structuring in the species, using both more markers and additional populations. To this end, we are currently sampling additional populations in South Africa, extending down to the southern edge of the distribution range of the species. These samples and the existing database will be screened using a larger range of microsatellite primers.

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References cited

Bowen, Brian W. 1998. What is wrong with ESUs?: the gap between evolutionary theory and conservation principles. Journal of Shellfish Research 17: 1355-1358.

Dracopoli, N.D., F.L. Brett, T.R. Turner, and C. J. Jolly. 1983. Patterns of genetic variability in serum proteins of the Kenyan vervet monkey *(Cercopithecus aethiops).* American Journal of Physical Anthropology 61:39-49. 1983.

Goodman, Simon J. 1997. RST CALC: A collection of computer programs for calculating unbiased estimates of genetic differentiation and determining their significance for microsatellite data. Molecular Ecology 6: 881-885.

Grobler, Paul J. and Jacob M. Matlala. 2002. Regional genetic variability among South African vervet monkey (*Chlorocebus aethiops*) populations. Acta Theriologica 47(2): 113-124.

Grobler, Paul J., Malan D. Pretorius, Karen Botha, Antoinette Kotze, Eric M. Hallerman, and Bettine Jansen van Vuuren. 2005. An exploratory analysis of geographic genetic variation in southern African nyala (*Tragelaphus angasii*). Mammalian Biology 70: 291-299.

Michalakis, Yannis and Lourent Excoffier. 1996. A genetic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. Genetics 142: 1061-1064.

Moritz, Craig. 1994. Defining "evolutionarily significant units" for conservation. Trends in Ecology and Evolution 9: 373-375.

Moritz, Craig. 2002. Strategies to protect biological diversity and the evolutionary processes that sustain it. Systematic Biology 51: 238-254.

Schneider, Stefan, David Roessli, and Lourent Excoffier. 2000. Arlequin ver. 2.000: A software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Turner, T.R. 1981. Blood protein variation in vervet monkeys (*Cercopithecus aethiops*). American Journal of Physical Anthropology 55:225-232.

Turner, T.R., J.P. Gray, F. Anapol, and M.L. Weiss. 2000. Genetics and morphology in vervet monkey evolution. American Journal of Physical Anthropology, Supplement 30: 305-306.

Vogler, Alfried P. and Robert DeSalle. 1994. Diagnosing units of conservation management. Conservation Biology 8: 354-363.

Waples, Robin S. 1995. Evolutionary significant units and the conservation of biological diversity under the endangered species act. American Fisheries Society Symposium 17: 8-27.

Weir, Bruce S. 1979. Inferences about linkage disequilibrium. Biometrics 35: 235-254.

Yeh, Francis C., Rong-cai Yang, and Tim Boyle. 1999. POPGENE ver. 3.31. Microsoft Window-based freeware for population genetic analysis.