Geographical genetic variability in vervet monkey (*Cercopithecus aethiops*) populations.

by

MOLOKO JACOB MATLALA

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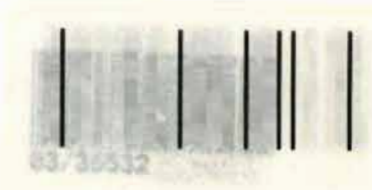
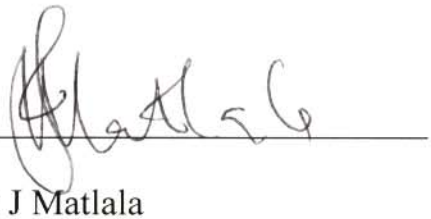


DECLARATION

I MOLOKO JACOB MATLALA declare that the work contained in this dissertation is entirely my own work and that all the sources I have used or quoted have been duly acknowledged by means of complete reference.

Signature: 

M J Matlala

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SUMMARY

A South African rehabilitation centre for illegally kept vervet monkeys required an evaluation of the genetic status of vervet monkeys, to determine whether animals from different geographical areas may be kept in the same enclosures and mixed during release back into the wild. Animals originating from three geographical regions (the former Transvaal, KwaZulu-Natal and the Eastern Cape) were studied using biochemical genetic and morphological approaches to address this question. The most prominent trend from allozyme data was derived from the locus **PRT-2** (an unspecified serum protein), where each of the three populations could be characterized by the absence or presence of unique alleles. A significant deviation of genotypes from Hardy-Weinberg equilibrium was found at the **PGD-1** locus in all the populations studied. Nevertheless, statistical coefficients indicated little genetic divergence, with genetic distance values of 0.001-0.003, gene flow values of 4.300 – 16.310 and an overall fixation index value of 0.046. Average heterozygosity did not differ appreciably among populations (2.5-3.3%). The morphological study identified suitable traits, free from the influence of growth allometry, which can be used for inter-population comparisons. No significant morphological differences between conspecific populations were however found. It is concluded that vervet monkeys from the species' wider distribution range is relatively monotypic, but that monkeys from different geographical areas should not be unduly mixed, pending the results of finer grained molecular studies.

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INTRODUCTION
CHAPTER ONE

CONTENTS:

1.1. RATIONALE FOR A GENETIC SURVEY OF VERVET MONKEYS.

Young vervet monkeys are often kept as pets by South African families. Captured young vervet monkeys are nurtured in a somewhat unnatural environment where they are deprived of the social structure of a natural troop of monkeys, leading to behavioural problems. The practice of keeping monkeys as pets is illegal and it usually culminates in the monkeys being confiscated by Nature Conservation authorities. The seized animals are either destroyed or, more recently, transferred to rehabilitation centres, such as the Riverside Wildlife Rehabilitation Centre at Letsitele and the Arthur Hunt Wildlife Rehabilitation Centre near Tzaneen (both in the Northern Province). The rehabilitation centres currently house at least 600 vervet monkeys originating from various regions of South Africa.

At present, because of some uncertainty pertaining to the status of subspecies, Nature Conservation authorities are not allowing the mixing of monkeys originating from different regions in enclosures, nor the release of rehabilitated monkeys. The release could result in genetic pollution if different taxonomic groups were involved (see section 1.1.1). The managers of wildlife rehabilitation centres are required to obtain permits from conservation authorities. The Department of Environmental Affairs and Tourism issued the first permits to rehabilitation centres on 01 November 1997, after the latter have been in operation for at least two years. There are special conditions under which the permits to

keep and rehabilitate vervet monkeys are issued by the Department of Environmental Affairs and Tourism.

- The permit does not absolve the holder from the necessity of obtaining such other permits and documents as may be required by law from the relevant province or country.
- The permit can be withdrawn by the Chief Directorate: Environment if the holder thereof fails to comply with the conditions thereof.
- All individuals have to be microchipped.
- Sub-species are to be kept separately. No cross-breeding is to take place.
- The Department of Environment has to be consulted before any releases.
- The permit may be withdrawn at any time should the validity thereof be in contravention with the national policy, once it is ratified.
- A register must be kept of all mortalities and natalities. The register is subject to inspections from nature conservators appointed by the chief directorate of Environmental Affairs, North Province.
- No claim may be instituted against the chief directorate, Environmental Affairs, Northern Province for any losses or expenditure incurred should the new national policy prohibit the continuation of the practise for which this centre was developed. The continuation of the practise is thus conducted at own risk.
- All animals shall be disposed of in a manner prescribed by our office should the functioning of the centre be discontinued for any reason whatsoever.
- The permit is temporary. The holder of this permit must apply in writing for renewal two months prior to the expiry date of the permit.

- A permit is needed to import into the province, export therefrom or convey therein a vervet monkey. A permit is also needed to sell, keep or donate a vervet monkey.

1.1.1. Regional genetic variation.

In recent years, genetic management has grown in prominence to establish itself as an integral component of wildlife management and conservation strategies. Genetic considerations are a prerequisite for the management of populations both in captivity and in the wild (Spellerberg, 1996a). For this reason, local conservation bodies are highly conscious of the importance of genetic management. An important aspect of such management is the conservation of distinct genetic variants within species because these genetic variants form the foundation for biological diversity. Genetic diversity is of major importance to all levels of biological diversity and has been the basis of evolutionary processes. Within populations of species genetic diversity brings about a variety in shape, colour, behaviour, resistance to disease and tolerance to adverse conditions. In essence, genetic diversity is important for populations if they are to survive changes in the environment, pathogens and parasites. For these reasons, it is also a high priority of the 1992 Convention of Biological Diversity, of which South Africa is a signatory (Glowka *et al.*, 1994; McNeely, 1996; O'Connell, 1996; Spellerberg, 1996a; b; and Worley, 1996). The convention contains three fundamental obligations for its signatories:

- conservation of biodiversity;
- sustainable use of natural resources; and
- co-operative sharing of biotechnology and the benefits of biodiversity.

The first obligation (conservation of biodiversity, including regional genetic variability) is of particular relevance to the genetic management of captive vervet monkey populations. Two opposing arguments pertaining to conservation and genetic divergence can be made, as discussed below.

1.1.1.1. Rigid preservation of geographical genetic variants.

When a species occur over a relatively wide distribution area, it is possible that populations found at extreme ends of the range will have diverged genetically to some extent. If genetic divergence is of significant magnitude, it may be prudent to prevent the subsequent mixing of animals from such groups. Templeton (1986); Schmidt and Engstrom (1994); Schmitt and Tomiuk (1994); and Gray (1996) stated that where local populations may have diverged genetically and evolved internal coadapted combinations of genes, a reduction of fitness may be observed in the progeny as the two populations might have fixed alternative alleles adapted to specific environmental circumstances. This phenomenon is also known as 'outbreeding depression'.

Genetic studies on wild populations of cercopithecid species have revealed complex patterns in their population dynamics and corresponding genetic population structure (Schmitt and Tomiuk, 1994). There is a remarkably high degree of diversity in wild populations within this group, and notable genetic variation has been observed between and even within local natural populations (Nozawa *et al.*, 1977; Palmour *et al.*, 1980; Kawamoto and Ischak, 1981; Turner, 1981; Nozawa *et al.*, 1982; Kawamoto *et al.*, 1984). Genetic divergence due to geographical distribution is well known (Ryman *et al.*,

1980) and can be regarded as a natural process leading to speciation via differential selective pressures. Such viable conspecific populations should not be unduly transposed but rather their ability to change and adapt on ecological and evolutionary time scales should be conserved. Chesser *et al.* (1982) reported that genetic differences exist over much smaller physical distances than previously thought, with significant implications for the design of management programmes. There is thus a strong need for more detailed studies examining the spatial distribution of genetic variation, in particular to determine on how fine a geographical scale such a heterogeneity between populations may exist to regulate translocation and mixing of animals.

1.1.1.2. Free interbreeding of geographical genetic variants.

It is conceivable that a species may occur over a wide geographical area, without any significant divergence developing. There are many mammal species that do not show pronounced differences although they occur over wide distribution areas. In addition, even if small genetic differences between conspecific populations should be found, they are often the result of temporal divergence. Spatial and temporal scales should also be considered when examining the genetic structure of populations. According to Gaines *et al.* (1997), studies of genetic structure done as snapshots in time may lead to erroneous results. Such presumed divergence may not be significant and it could be nullified following the resumption of inter-population gene flow (Grobler, 1995). If such a condition prevails, it would be irrational to prevent mixing between populations and artificial separation may even be counter-productive to the overall goal of genetic management if it results in induced inbreeding in local populations.

1.1.1.3. A genetic survey of captive vervet monkeys.

The question as to whether genetic divergence should be inferred from geographical separation can be resolved with the aid of biochemical and molecular genetic techniques such as allozyme electrophoresis, Random Amplified Polymorphic DNA (RAPDs) and microsatellites. Allozyme electrophoresis was chosen as the best approach to use during the current study, with its well-established record of ease of application, relatively low cost, proven repeatability and ease of statistical interpretation. However, allozyme variation may not be representative of total genetic variation, hence recommendations for genetic conservation based solely on allozyme data may not be totally accurate (Hamrick, 1983). It is therefore recommended that data inferred from allozyme electrophoresis be complemented with data from other molecular genetic techniques or even morphological data. Development of an efficient strategy for preservation requires sets of genetic markers that characterise distinct populations (Kemp and Teale, 1994). Acquiring the information necessary to make correct decisions is part of the wildlife manager's task (Bailey, 1984). The current study provides an opportunity to apply laboratory techniques in molecular genetics to address a problem that is of real concern to Conservation authorities in the Northern Province.

Genetic information of vervet monkeys is also important because such information can be used to define the distinctiveness of specific populations or even define the pedigrees of individual animals (how related monkeys within populations are). The need for genetic information is high due to the fact that genetic resource banks of frozen or fixed somatic cells from wild species is not yet in operation on a larger scale, especially in South Africa.

In the future, there might be the need to store germplasm of most wild animal species, including vervet monkeys, for conservation, medical purposes or advancement of science and for it to succeed molecular screening would be needed to enable a high proportion of genetic diversity to be preserved. The storage of germplasm from animals of known provenance, which have been genetically characterised by biochemical or molecular markers, can secure the integrity of a gene pool against the threat of introgression (Holt *et al.*, 1996).

1.2. CLASSIFICATION, DISTRIBUTION AND DISCRIPTION OF VERVET MONKEYS.

The vervet monkey (*Cercopithecus aethiops*); samango monkey (*Cercopithecus mitis*) and chacma baboon (*Papio ursinus*) represent the subfamily Cercopithecinae in South Africa. All the long-tailed monkeys of Southern Africa including the vervet monkey (*C. aethiops*) have been known by the generic name *Cercopithecus* Linnaeus, 1758 for many years. Recently, the vervet monkey was given the generic name *Chlorocebus* and there is a consensus reached to revert back to *Cercopithecus* (Groves, 1993). Meester *et al.* (1986) listed six subspecies of vervet monkey from the sub-region. Two sub-species occur in South Africa namely *C. a. pygerythrus* from the southern and eastern Cape Province and Natal, and *C. a. cloetei* from northern KwaZulu Natal and the western Transvaal.

Vervet monkeys occur in northern, north-eastern and southern Namibia, in the northern, eastern and in parts of south-eastern Botswana, the former Transvaal, Swaziland,

Mozambique, south of the Zambesi river, the former Natal, the western Free State, in the eastern, southern and along the Orange and Vaal rivers in the Cape Province, where they occur coastally as far west as the George and Knysna districts (Skinner and Smithers, 1990).

The vervet monkey is a woodland species (Skinner and Smithers, 1990). It was found that the vervet monkey and samango monkeys have somewhat of an overlap in their patterns of space and resource utilisation where they occurred sympatrically (Moreno-Black and Maples, 1977). *Cercopithecus aethiops* is identifiable with its pure black face, grizzled greyish upper parts, with tail the same colour as the body or slightly darker, the tip blackish, patch of reddish hair under the root of the tail in adult males, hands and feet predominantly black, and outer surface of the arms not black (Skinner and Smithers, 1990).

Adult males have the powder-blue scrotum that is a characteristic feature of the genus and this allows identification of the adult male (Henzi, 1981; 1985). A dermal deposition of melanin in melanocytes has been confirmed to account for the blue coloration based on observations by Price *et al.* (1976). However, Price et al. (1976) mentioned that blueness of the scrotal skin is modulated by the state of hydration of the dermis. It clearly implies that more studies should be done on scrotal skin because it is not yet understood how this hydration is regulated.

The skull has a rostrum, which is not as pronounced as found in the genus *Papio*. The rostrum slopes evenly forward from the top of the sockets to the front of the skull. The eye sockets tend to be flattened on their upper margins and separated by a bony septum that broadens out slightly to the front of the nasal openings. The canines are sharp-pointed and the upper canine has a sharp edge on the posterior surface that is kept sharp by occlusion on the elongated first premolar in the lower jaw (Skinner and Smithers, 1990).

Male vervet monkeys disperse non-randomly in the company of their brothers from their natal groups at sexual maturity, migrating to neighbouring groups, this activity peaking during the mating season (Henzi and Lucas, 1980; Cheney and Seyfarth, 1983). This benefits young males by minimising the risk of predation or reducing the probability of attack by resident males and females. Older males transfer randomly and alone to groups that are more distant. This appears to have important genetic consequences for the population as a whole, avoiding the negative effects of excessive endogamy.

Vervet monkeys around tourist areas can be a major attraction for visitors to game parks. The vervet monkey is however also considered a pest species in many quarters. Vervet monkeys are usually found around lodges, settlements, and garbage disposal areas even in some suburbs, for example in Durban (Lee *et al.*, 1986). These monkeys have ample access to water and food to supplement their natural diets. These conditions cause population size to increase primarily due to a high birth rate. Rowell and Richards (1979) pointed out that vervet monkeys in most natural areas are seasonal breeders, but the births among the groups in captivity and next to lodges, settlements and garbage disposal areas

do not strictly follow seasonal pattern. The 'off season' breeding may be the result of high levels of nutrition achieved by access to garbage throughout the year (Lee *et al.*, 1986). Conflict with humans is normally brought about by the vervets' tendency to break into cottages, kitchens and cars to obtain food. They are also infamous for attacking and seriously biting tourists and members of local communities.

1.3. AIMS AND OBJECTIVES.

The aims of this study are as follows:

- to establish a database on the distribution of biochemical genetic variability in South African vervet monkey populations;
- to use this information in order to find out whether vervet monkeys originating from different regions can be mixed or not; and
- to ascertain what extent morphological information can supplement biochemical genetic information.

These aims will be addressed using biochemical genetic methods and morphological analyses to study captive vervet monkeys originating from diverse geographical regions.

SAMPLING SITE AND SAMPLING
CHAPTER TWO

The Riverside Rehabilitation Centre, South Africa (23^0 51'S; 30^0 24'E; see Figure 2.1), hosts several hundred vervet monkeys originating from various regions of the country. The regions of origin of monkeys can be broadly classified as "Former Transvaal" (based on the now defunct political region), "KwaZulu-Natal" and "Eastern Cape" (Figure 2.1). Sampling occurred during a period from January 1998 through October 1998. Vervet monkeys originating from these three geographical regions are kept in separate enclosures to ensure that there is no interbreeding among different populations (Figure 2.2), in line with the conditions imposed by conservation authorities. A comprehensive database stipulating the origin, age, identity, weight and other morphological measurements of each monkey is kept and updated every three to five weeks by the personnel working at the Riverside Wildlife Rehabilitation Centre.

The sample sizes obtained comprised of 25 individuals originating from Kwazulu-Natal, 25 from the former Transvaal, and 19 individuals from the Eastern Cape. The monkeys were caught from the enclosures with a net and sedated with an intra-muscular injection of ketamine hydrochloride at a dose of 5.0 mg/kg. The injected region was gently rubbed to avoid swelling and this also helped to spread the sedative evenly. The monkeys were unconscious for approximately 45 minutes.

For the taking of blood samples for genetic analysis (described in Chapter 3), a region on the upper leg where the femoral vein was clearly discernible was selected, the hair shaved and the region sterilized with 70% ethanol. The leg was chosen because it is problematic to draw blood from the jugular veins of small primates. A rubber band was placed around

the upper leg to allow blood to accumulate in the vein. Approximately 5 ml of blood was drawn from the femoral vein of each monkey using Terumo' syringe and the rubber band was removed as soon as the needle was inserted (Figure 2.3). An antiseptic was applied on the region where blood was drawn to avoid a possible microbial infection and to facilitate healing of the wound. The monkeys were then put in a small cage to recover before they were released back into the bigger enclosures.

Most of the monkeys already had implanted microchips to facilitate easy identification and this is also in line with special conditions under which the permit is issued by the Department of Environmental Affairs. The microchip number, name of the monkey (where applicable), geographical origin, morphological measurements, sex, and age of each monkey from which blood was collected were recorded. Monkeys without identity numbers were inserted with microchips to avoid drawing blood from the same monkeys on subsequent research trips. Measurements for the morphological study (Chapter 4) were taken using veneer calipers, measuring tape (to nearest mm) and scale. The morphological parameters used will be discussed in Chapter 4.

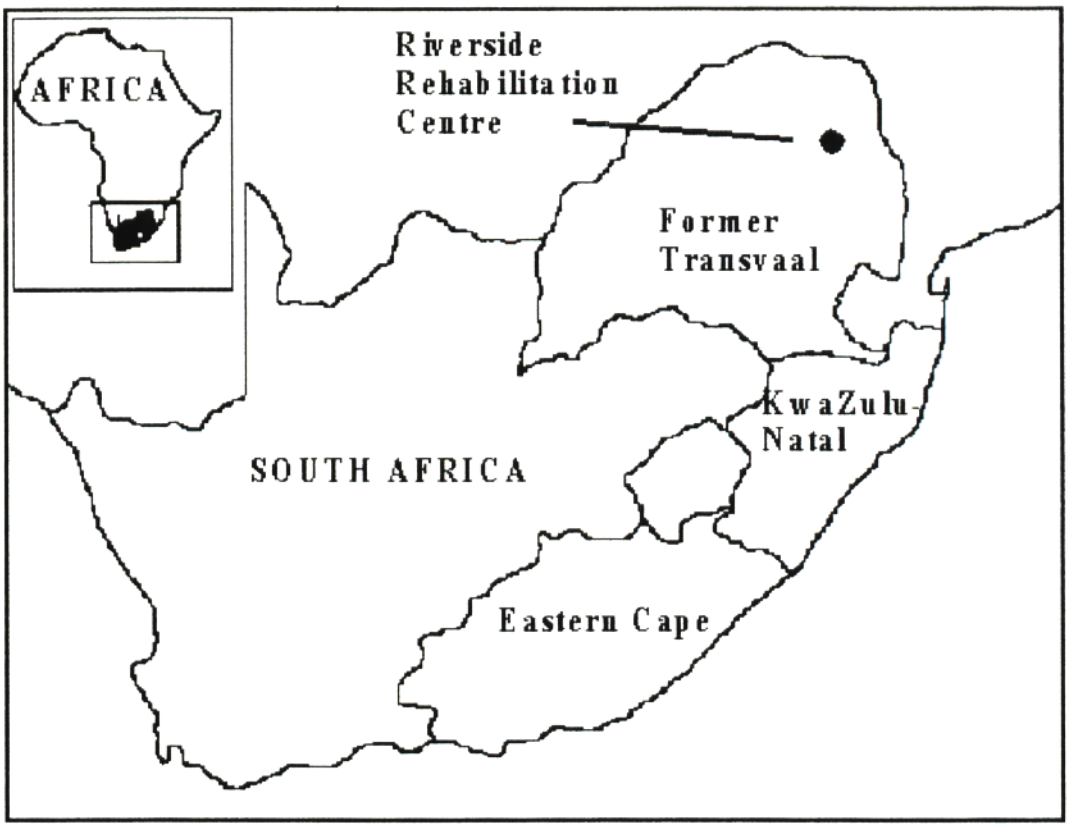

Figure 2.1. The location of the Riverside Wildlife Rehabilitation Centre, near Letsitele in the Northern Province.

Figure 2.2. An enclosure with vervet monkeys originating from Natal.

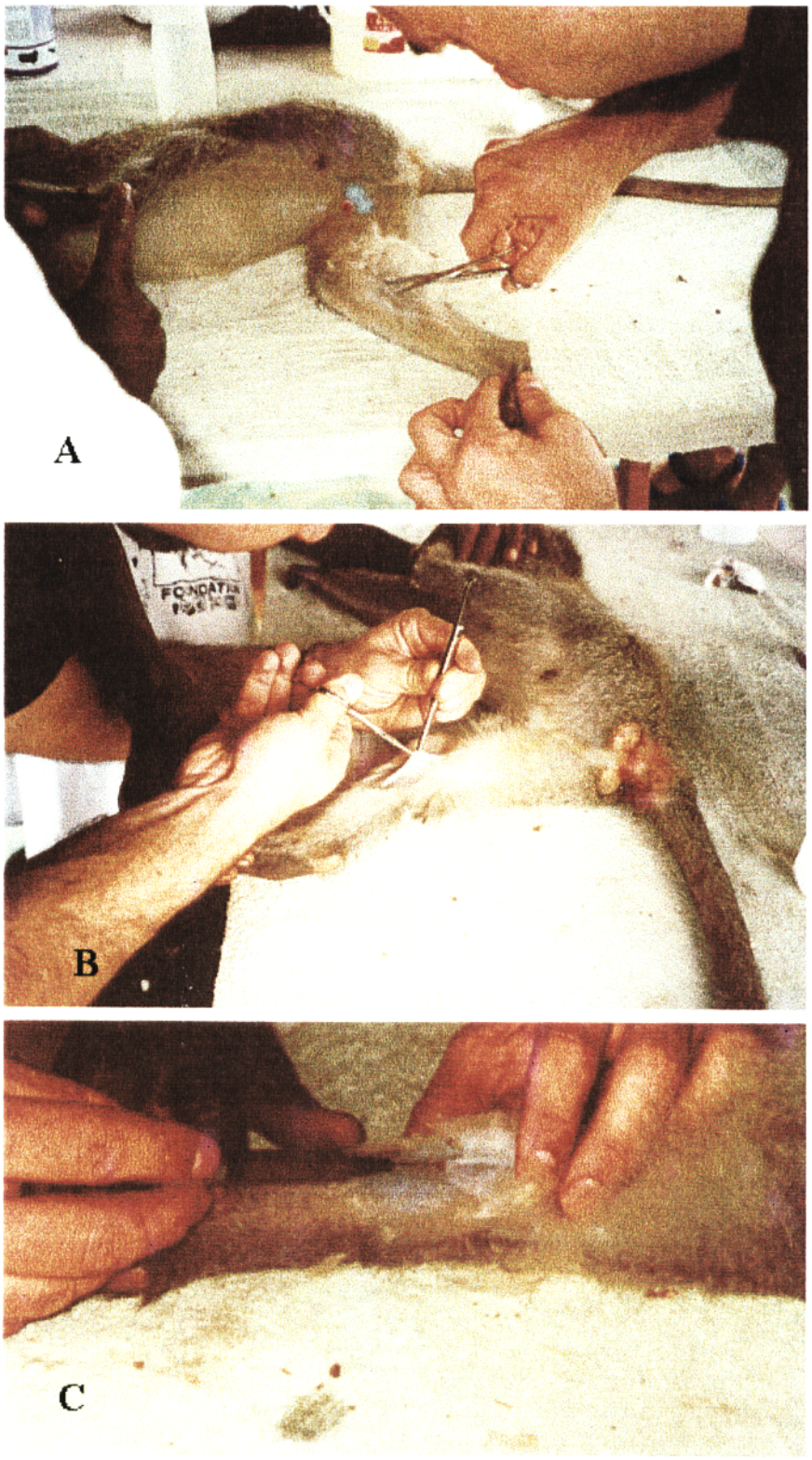

Figure 2.3. The upper leg is cleaned and shaven (A), and the skin opened (B) before blood is drawn directly from the femoral vein of a vervet monkey (C).

GENETIC ANALYSIS
CHAPTER THREE

CONTENTS:

3.1. INTRODUCTION – RATIONALE FOR THE USE OF ALLOZYME ELECTROPHORESIS.

Allozyme electrophoresis has provided the most complete picture of the comparative genetic diversity of different animal and plant species (Gray, 1996). The technique has also generated information that has been very useful for understanding population processes in nature (Grant, 1989). This is because allozyme electrophoresis is relatively inexpensive and easy to use (Hartl, 1980; Adams 1983; Ayala, 1983; Hamrick, 1983; Powell, 1983; Liu and Furnier, 1993 and Gray, 1996). Many samples (usually 30 or more) can be run on one gel, and from one gel there can be four slabs for staining. For this reason it is possible to assess variation in a large number of individuals in a comparatively short time (Gray, 1996).

Some researchers are of the opinion that allozyme electrophoresis detects only a proportion, perhaps less than a third of the total underlying genetic variation (Chambers, 1983; Powell, 1983; Grant, 1989; Liu and Furnier, 1993 and Gray, 1996). The argument of these authors is based on the fact that there are different sets of genes, not all detectable by allozyme methods. The first set of genes codes directly for proteins with structural or enzymatic functions and they are called structural genes. The second set of genes does not specify structural proteins or enzyme and those genes are called regulatory genes. The latter genes bring about variation that cannot be detected by allozyme electrophoresis. It is also possible that some amino acid replacements may not change the charge or configuration of a protein molecule (Grant, 1989).

Nevertheless, allozyme electrophoresis is a useful tool in that in species it enables the calculation of coefficients of genetic diversity and divergence. With key genetic parameters, a researcher is able to estimate how similar or different populations are. The researcher can move one step further and detect the genetic 'structure' of populations by calculating how their levels of diversity depart from those predicted by the preconceived model of random mating such as Hardy-Weinberg equilibrium. These measures are particularly important in conservation biology, especially in the context of endangered or rare species conservation (Frankel and Soule, 1981 and Gray, 1996). The present study utilizes genetic parameters derived from allozyme electrophoretic data in an attempt to resolve a problem of real concern in conservation.

3.2. MATERIALS AND METHODS

The vervet monkey populations from which blood samples were drawn were described in Chapter 2. Blood samples used for the analysis of specific proteins (electron-transfer method staining) and proteins whose substrates are bound to either α- or β- naphthol as found in esterase ('chemical reaction' stains), were frozen in liquid nitrogen (-196^{0}C) immediately after sampling and transported to the laboratory. Such samples were stored in labeled plastic sample collection tubes, with small holes pierced in the lids of the tubes to release pressure that might develop when the blood becomes frozen. Blood samples used for the analysis of non-specific proteins were stored in vacutainer sample tubes with lithium heparin to prevent clotting, and kept in an icebox. This was followed by separation of serum and blood cells by centrifugation at 1000 rpm for 10 minutes. After separation, both serum and blood cell samples were stored in a freezer at –20^{0}C.

3.2.1. Polyacrylamide gel electrophoresis (PAGE):

Serum proteins were examined by means of a horizontal polyacrylamide gradient gel system, following the procedures used at the accredited laboratory of the Animal Improvement Institute in Irene, Pretoria. The polyacrylamide gradient gel system consisted of a 12% resolving, 4% stacking, and 8% supporting gel. An in-house constructed mould was used for preparation of the gel. Gel casting is based on the combination of three solutions (Kotze, pers. com.).

Solution 1 was prepared using the following ingredients:

- 48g acrylamide
- 1.2g bis acrylamide
- 102ml of distilled water

The solution was filtered before use.

Solution 2, which was the gel buffer, was prepared using the following ingredients:

- 50ml tris (90.75g in 500ml distilled water)
- 50ml citric acid (20g in 500ml distilled water)
- 440µl temed.

Solution 3 was always prepared fresh using:

- 0.2g of ammonium persulphate in 100ml distilled water.

One liter solution of electrode buffer was prepared as follows:

- 80g of Tris
- 20g of Boric acid
- 650 µl Bromophenol

The resolving part of the gel (12%) consisted of solution 1, distilled water, solution 2, and solution 3 (at the ratio 3:1:2:2). The stacking (4%) and supporting (8%) parts of the gel consisted of similar components as the resolving part at the ratios 1:4:1:2 and 2:3:1:2 respectively. Into the mould, solutions for the resolving (12%) part of the gel were firstly poured and allowed to set for 15 minutes. Solutions for the stacking (4%) part of the gel were then poured and allowed 45 minutes to set. Finally, solutions for the supporting (8%) part of the gel were poured and allowed to set for 30 minutes.

Serum samples were loaded onto the stacking (4%) part of the gel using wicks of absorbent paper, which were then removed after 15 minutes. Excess fluid of serum was removed by blotting briefly on paper toweling. The electrode buffer was poured in the electrode vessel reservoirs and this formed a discontinuous buffer system. The PAGE was carried out at 350v/cm for 4 - 5 hours.

3.2.2. Starch gel electrophoresis:

Sample preparation, gel and buffer composition, electrophoretic separation of gene products and staining followed standard methods such as summerised in Harris and Hopkinson (1976), Ward (1977), Grant (1989), and Murphy *et al.* (1990). A series of test

gels was run to ascertain which buffers provided the best results for selected enzymes. Three buffer systems were used, namely:

- **RW** (a discontinuous buffer system with Tris-citric acid gel buffer, and lithium hydroxide - boric acid electrode buffer),
- **TC** (a continuous buffer system with Tris and citric acid in the sample gel and electrode vessel reservoirs), and
- **MF** (a continuous buffer with Tris, boric acid and EDTA in the sample gel and electrode vessel reservoirs).

Starch gels (13%) were run for 2.5 – 3.0 hours at 45mA for both **RW** (Ridgway *et al.*, 1970) and **MF** (Markert and Faulhaber, 1965). Hydrolysed starch gel (13%) for **TC** (Whitt, 1970) was run for 3.5 – 4.0 hours at 45mA. A recirculating cooling system and plate was used to cool the starch gels during electrophoresis.

Before staining, the gels were sliced into four slabs using a thin wire while supported by an L-shaped cutting board to prevent the gels from slipping off. During cutting a glass plate was also placed on top to support the slabs. The top slice, which usually has a dry surface, was inverted before staining. Non-specific protein dye (Coomassie blue) was used to stain for general or unspecified proteins (**PRT**). A 'chemical reaction' stain was used to resolve esterase (**EST**). Electron transfer dyes mixed with 2% agar overlays were used to stain the remaining proteins.

3.2.3. Genetic interpretation and nomenclature:

Genetic interpretation of gels and nomenclature followed the methods described by Murphy *et al.* (1990) and Shaklee *et al.* (1990). The number and position of the stained bands on the gels were interpreted as the genotype at the gene locus coding for the enzyme (Ayala, 1982). All differences in electrophoretic mobility were assumed to be of genetic origin and inherited in Mendelian fashion. From the stained bands it was assumed that the electrophoretically detectable variants differ genetically from each other and they were referred to as alleles (Gutierrez *et al.*, 1983). The alleles were labeled according to their relative mobilities. Loci were designated with numbers, starting from the cathodal end of the gel. Since starch gels were run horizontally, they were viewed with the origin down (towards the viewer). The loci cathodal to the origin were indicated by a minus sign according to Avise *et al.* (1980). The statistical analysis of allozyme data was done by the use of the computer programme BIOSYS-1 (Swofford and Selander, 1981; 1989).

3.2.4. Measures of genetic variability:

- Proportion of polymorphic loci or polymorphism (**PPL**):

One of the ways to measure the amount of genetic variation in a sample of individuals is to calculate PPL. For this study a locus was considered polymorphic when the most common allele has a genotypic frequency of at most 0.95 (Ayala, 1982 and Grant, 1989). **PPL** (Nei 1978) was calculated as:

$$\mathbf{PPL} = x / r$$

where x is the number of polymorphic loci in a sample and r is the total number of loci sampled.

The use of the proportion of polymorphic loci to measure genetic variability has the drawback in that it does not distinguish between highly polymorphic loci and those with low levels of polymorphism (Grant, 1989). As a result, it is somewhat arbitrary in that it changes substantially depending on the criterion used for samples with only low frequency variation at each locus.

- Average heterozygosity (**H**):

Average heterozygosity is the most commonly used measure of genetic variability when analysing allozyme-based data. In the present study **H** was calculated as an estimate based on Hardy-Weinberg expectations and as an unbiased estimate based on conditional expectations (Levene, 1949; Nei, 1978). It is a biologically meaningful parameter because diploid individuals in a population are either homozygous or heterozygous (Grant, 1989). **H** (Nei 1975) is the average of heterozygosity for a locus over all loci, including monomorphic loci.

$$\mathbf{H} = \Sigma^{\text{loci}} (1 - \Sigma^{\text{allele}} P_{ij}^{2}) / (\text{No.Loci})$$

where the $\underline{P}_{ij}$ are the genotypic frequencies of genotypes and where $\underline{i}$ is not equal to j

- Average number of alleles per locus (**A**):

The amount of variability at a locus can be measured by **A** (Nei 1978). There are two drawbacks associated with this measure. The first is that it is heavily dependent on sample sizes. Secondly, a simple counting of alleles does not take into account the frequencies of the alleles at a locus.

3.2.5. Relative measures of population differentiation:

Allelic frequencies in isolated populations tend to change over time, because of random changes in populations due to the chance selection of gametes producing frequencies that differ to some extent from the previous generation (Grant, 1989). This can be tested for by using chi-square test for deviation from Hardy-Weinberg equilibrium in each population and contingency chi-square analysis at all polymorphic loci following Levene (1949). Population variation in the present study was measured by using fixation indices ($\mathbf{F_{ST}}$, $\mathbf{F_{IT}}$ and $\mathbf{F_{IS}}$), which are used to describe genetic differentiation between populations. In $\mathbf{F_{ST}}$, "**S**" stands for subpopulation and "**T**" the total population (Wright, 1965 and 1978). It is computed from allele frequency data by:

$$\mathbf{F_{ST}} = \sigma^2 / p\,(1 - p)$$

where σ^2 is the variance of allele frequencies among populations and $p\,(1 - p)$ is the theoretical maximum variance of the average allele frequency, p.

$\mathbf{F_{IT}}$ indicates the amount of inbreeding in the population due to the population subdivision whereas $\mathbf{F_{IS}}$ indicates the degree of allelic fixation in individuals relative to the subpopulation (Nei, 1986).

3.2.6. Genetic distances:

Several measures of genetic distance using electrophoretic data have been devised, but Nei's (1972) coefficients of identity (**I**) and distance (**D**) are used most frequently (Grant, 1989). In the present study, genetic distance (**D**) and a dendrogram illustrating relationships among populations were determined using BIOSYS-1, the methods

described by Nei (1972) and unweighted pair-group method with arithmetic averaging, UPGMA (Swofford and Selander, 1981).

3.3. RESULTS

Proteins stained for, loci resolved, enzyme commission numbers (E.C.), and optimum buffer type for each protein stained for are summerised in Table 3.1. A total of twenty-six loci were resolved, of which three displayed polymorphism. The polymorphic loci were mannose-6-phosphate isomerase (**MPI-1**), 6-phosphogluconate dehydrogenase (**PGD-1**), and general protein (designated **PRT-2**). The mannose-6-phosphate isomerase locus encodes monomeric enzymes, which implies that the enzymes consist of a single peptide chain in its active form. The monomeric enzymes display a single band for homozygotes and two bands each with equal intensity for heterozygotes. The 6-phosphogluconate dehydrogenase locus encodes dimeric enzymes, which have more than a single subunit in their active form. Heterozygous phenotypes of dimeric enzymes have a banding density ratio of 1:2:1 with the center heteromeric band of the same intensity as the band representing the homozygote. Relative mobilities of alleles, genetic diversity coefficients and allelic frequencies for polymorphic loci are presented in Table 3.2. The highest average heterozygosity (**H** = 3.3%) was found in the Eastern Cape vervet monkeys. The Transvaal vervet population showed the lowest average heterozygosity (**H** = 2.5%) and the Natal population possessed an intermediate heterozygosity value of 2.9 %. The Natal and Eastern Cape vervet populations share the same value of proportion of polymorphic loci (**PPL** = 11.54%), with a lower value of 6.79% in the Transvaal population.

Two vervet monkey populations studied had specific alleles at the **PRT-2** locus. One allele, **PRT-2*100** occurred in all populations. The KwaZulu-Natal vervet monkey population has an allele (**PRT-2*93**) cathodal to the most common allele, which was not observed in the other populations. Similarly, the Eastern Cape vervet monkey population has a unique allele (**PRT-2*114**), anodal to the common allele at **PRT-2**, which was not found in either of the Transvaal or KwaZulu-Natal populations

Chi-square goodness-of-fit test showed that a significant deviation of genotypes from Hardy-Weinberg equilibrium was found at the **PGD-1** locus in all the populations studied ($p < 0.013$ in the Transvaal population, $p < 0.004$ in the Kwazulu-Natal population, and $p < 0.001$ in the Eastern Cape). There was a significant deviation from Hardy-Weinberg equilibrium at the **PRT-2** locus only in the Natal vervet population (Table 3.3). Overall contingency chi-square analysis of allele frequencies at all loci (Table 3.4) showed no significant differences between populations from different geographical regions ($p < 0.224$ for **MPI-1**, $p < 0.298$ for **PGD-1**, and $p < 0.086$ for **PRT-2**). Pair-wise contingency chi-square analysis of allele frequencies at all loci (Table 3.5) also showed no significant differences between populations from different geographical regions.

The average $\mathbf{F_{ST}}$ value of 0.046 obtained in the present study confirms that most diversity resides within rather than between vervet monkey populations, as expected for conspecific populations (Table 3.6). Further, pair-wise, analysis of F_{ST} values confirmed that most diversity resides within rather than between vervet monkey populations (Table 3.7). The gene flow was more positive between the Transvaal and KwaZulu Natal

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populations (N_{em} = 16.310) than between the Transvaal and Eastern Cape populations with N_{em} value of 4.300 (Table 3.7), with an intermediate level of gene flow (N_{em} = 7.266) between the KwaZulu Natal and Eastern Cape populations. Genetic distance values were comparatively insignificant and ranged from 0.001 – 0.003. The KwaZulu-Natal population showed a very close identity to the Transvaal vervet monkey population with a genetic distance value of 0.001 (Table 3.8). Genetic distance (**D** = 0.003) between Eastern Cape and Transvaal populations reflected the greater geographical distance separating these two populations, with an intermediate genetic distance of 0.002 between the KwaZulu-Natal and Eastern Cape populations.

3.4. DISCUSSION

3.4.1. Genetic divergence:

The most significant observation from the allozymic results was found at the **PRT-2** locus, where all three populations showed the absence or presence of unique alleles. One allele at this locus (**PRT-2*100**) occurred in all populations, but **PRT-2*114** is unique to the Eastern Cape region and **PRT-2*93** was found only in the KwaZulu-Natal population. This potentially provides a hope in finding distinct set of markers to differentiate among regional South African vervet monkey populations, and suggests that the reservations about translocations, expressed by conservation authorities, were not unfounded.

Statistically, the divergence among geographical populations is less pronounced. The small genetic distance values (**D** = 0.001 to 0.003) and high gene flow values ($\mathbf{N_{em}}$ =

4.300 to 16.310) found in the present study show that in the wild, gene flow is maintained across the distribution range of vervet monkeys. The genetic distances found are also within the expected range for conspecific populations (Thorpe, 1982). However, it has been reported that there may be basic differences in the amount of genetic divergence between certain groups of species and this has been confirmed by smaller genetic distances between species of birds as compared to genetic distances between pairs of species of reptiles (Grant, 1989). This can be attributed to the fact that bird species are simply over-described on the basis of plumage. It is therefore important to calibrate genetic distances for each major high taxon.

The genetic distance values found in the present study are comparable to the mean genetic distance (0.004) found between neighbouring troops of the crab-eating macaque, which fall under the same subfamily as the vervet monkey (Kawamoto *et al.*, 1984). The genetic distance values calculated during the present study are however much lower than the inter-population genetic distance value of 0.1059 reported in the crab-eating macaque and the high values of 0.0985 – 0.1218 between rhesus and Japanese macaques, which belong to different species (Nozawa *et al.*, 1977).

Although the magnitude of **D** values calculated was very small, it is notable that the relative genetic distances do reflect the geographical distribution of the populations, with the Transvaal and Eastern Cape populations, at the extreme ends of the study area, also separated by the largest **D** value (0.003). Vervet monkeys from adjoining areas are correspondingly less diverged, with **D** values of 0.001 between the populations from the

former Transvaal and KwaZulu-Natal, and with **D**= 0.002 between the latter population and monkeys from the neighboring Eastern Cape.

The average $\mathbf{F_{ST}}$ value of 0.046 obtained in this study suggests nothing to little genetic divergence between populations. Turner (1981) estimated an $\mathbf{F_{ST}}$ value of 0.062 in seven groups of vervet monkeys from the Awash National Park in Ethiopia, and commented that even this higher value indicated the lack of differentiation between vervet monkey groups. Wright (1978) categorized $\mathbf{F_{ST}}$ values below 0.05, as found during the current study, as suggestive of "little genetic differentiation".

The aforementioned suggests that there is little random genetic drift in natural populations of vervet monkeys. This confirms the prediction that males probably maintain gene flow across a wide distribution range through migration. Divergence that may occur at extreme ends of the range of vervet monkeys is nullified following the frequent resumption of inter-troop gene flow brought about by male migration. The two $\mathbf{F_{ST}}$ values from Ethiopian and South African populations confirm that most diversity in this African cercopithecid species resides within rather than between monkey populations. The weighted average value for all polymorphic loci of $\mathbf{F_{IS}}$ between geographic groups (0.436) is high and this implies a below average amount of non-random mating occurring within each of the geographic groups. When $\mathbf{F_{IS}}$ is close to zero, it indicates that there is high random mating. The mean $\mathbf{F_{IT}}$ value of 0.462 is relatively high, suggesting some barriers to optimal gene flow (see also section 3.4.2).

3.4.2. Genetic diversity:

An analysis of levels of genetic diversity was not considered a priority during this study. Since the vast majority of the monkeys studied are first generation captive animals, the level of heterozygosity at Riverside should be representative of levels in wild populations. Of all South African mammal species, the movements of vervet monkeys are probably among the least affected by human developments, and they occur in large numbers; factors which should ensure healthy levels of genetic diversity.

There were no real differences among heterozygosity levels from the regional populations, with 2.5% in the former Transvaal group, 2.9% in KwaZulu-Natal and 3.3% in monkeys from the Eastern Cape. Polymorphism in the KwaZulu-Natal and Eastern Cape populations (12%) is slightly higher than the value of 8% recorded in monkeys from the former Transvaal. This is due to fixation for a single allele at the **PRT-2** locus in the latter population, with the KwaZulu-Natal and Eastern Cape populations each displaying an additional and unique allele at this locus, as discussed above.

The results of this study thus confirm the hypothesis of relatively less retained heterozygosity, with values of 2.5-3.3%. These values are only slightly lower than **H** values reported for a vervet monkey population of Kenyan origin (3.5%) by Schmitt and Tomiuk (1994). Turner (1981) estimated higher average heterozygosity values of 5.6% in the vervet monkeys from the Awash National Park in Ethiopia. This difference could be geographically based or be reflective of the different loci sets used during the latter survey and the present study. Turner (1981) used 23 protein coding loci, a number which

appears comparable to the 26 used during the current study, but in fact only nine loci were common to both studies. The value estimated for vervet monkeys during the current study is however similar to or higher compared to typical values estimated for several other local mammal species using well established and comparable locus sets, e.g. 2.14-4.3% in blue wildebeest (Grobler and Van der Bank, 1993), 1.53-3.78% in sable antelope (Grobler and Van der Bank, 1994b) and 1.8-2.0% in springbok (Grobler *et al.*, 1999).

Relatively high levels of genetic diversity have been estimated for other species of the sub-family Cercopithecinae (to which vervet monkeys belong), compared to the **H**=2.5-3.3% found during the current study. Kawamoto *et al.* (1984) found average heterozygosity values, which ranged from 2.46% to 5.66% in five troops of the crab-eating macaque (*Macaca fascicularis*). The lowest heterozygosity value of 2.46% was found in an isolated troop inhabiting a small island. Shotake and Santiapillai (1982) reported very high genetic variability in toque macaques (*M. sinica*), with **H**=7.8%. Nozawa *et al.* (1982) reported the lowest genetic variability reported for macaques, in the Japanese macaque (*M. fuscata*) with an **H** value of 1.3%

It has been reported that only about one-third of the adult male population of vervet monkeys actually copulate with females (Struhsaker, 1967 and Soule, 1980). Gartlan and Brain (1968) have pointed out that in impoverished areas the peripheral male vervet monkeys gradually expand the feeding ranges leaving a troop of adult females, infants and juveniles with one or two adult males. Effective sizes of populations are a function of

the number of individuals actually contributing gametes to the next generation (Templeton and Read, 1983 and Wilcox, 1986). The difference between vervet monkeys and other species of the sub-family can be also attributed to both the small sample sizes used and the fact that the vervet monkey populations sampled do not reflect the whole genome of the species. Possingham (1996) stated that predicting the properties of very small populations using average values would very often have a high probability of inaccuracy because of small sample size. It should also be borne in mind that genetic diversity measured in any particular population is not a constant property of that population (Namkoong, 1983 and Spellerberg, 1996b). Genetic diversity is brought about by dynamic processes, both internally and externally. These processes (behaviour of the chromosomes at meiosis, behaviour of individuals in choosing mate and historical changes in population size) are continually changing genetic diversity.

Interestingly, significant ($p<0.001$ to 0.0013) deviations from expected Hardy-Weinberg genotypic frequencies were observed in four instances during the current study: for **PRT-2** in vervet monkeys from KwaZulu-Natal and for **PGD-1** in all populations. Turner (1981) reported that there was no deviation from random mating in the free-ranging vervet population of Awash National Park in Ethiopia. The deviations observed during the current study reflected a deficit of heterozygotes in all instances, which is surprising, considering that all the monkeys studied represent animals captured from large free-ranging populations.

Factors that might contribute to deviations from Hardy-Weinberg equilibrium include sampling error, mutation, selection, and migration. Sampling error (in this context) occurs because allele frequencies of the sample of gametes that form the zygotes of one generation may not be exactly representative of the allele frequencies of the parental generation that produced the gametes (Chambers, 1983). Both chromosomal and gene mutations can change allele frequencies although at a very slow rate. Mutation that has a slight effect on the phenotype can be of value to an organism and the value can be realised for the populations only by selection upon the gene pool (Campbell, 1974). Selection alters allele frequencies when some alleles are favoured at the expense of other alleles. Furthermore, migration of individuals into one from the other population may cause the allele composition of both populations to change (Harlt, 1980; Slatkin, 1980; 1981; 1982 and Allendorf, 1983).

3.4.3. Conclusion:

The genetic results derived from allozyme data present a dichotomy. Statistically, the results suggest that there is little motivation to continue to regard the populations sampled as "distinct" variants. The fixation index (0.046) and genetic distance (0.001-0.003) values obtained are below published estimates where no divergence between populations was inferred (Turner, 1981 and Kawamoto *et al.*, 1984). However, a biological, rather than statistical evaluation of the results suggests that the conservation of the distinct groups may indeed be prudent.

Allendorf (1986) demonstrated how a statistical coefficient (heterozygosity, in that instance), may not be the only way to describe processes of population genetics, and that allelic diversity should also be considered. Does the occurrence of unique alleles in two vervet monkey populations at a single locus provide sufficient motivation for continued separation? The rare alleles found in each of the KwaZulu-Natal and Eastern Cape populations occur at low frequencies of 0.1 in both cases. Monkeys from the former Transvaal do not have unique alleles but are characterised by the absence of specific alleles. Allendorf (1986) pointed out that low frequency alleles do not contribute much to the immediate response of a population to selection, but that the limit of response over many generations is determined by the initial allelic diversity present. The presence of rare alleles at a single locus *per se* does probably not warrant classification of distinct variants, but may be sufficient evidence to suggest that additional region-specific markers may exist. It is suggested that this finding provides the necessary motivation to maintain the *status quo* of non-mixing at the Riverside facility until a more comprehensive study, based on finer grained genetic markers such as microsatellites, can be implemented or an increase of samples.

3.5. APPENDIX.

Table 3.1. Proteins stained for, E.C. number, loci resolved and most suitable buffer for each locus.

Protein	E. C. no.	Loci resolved	Buffer
Alcohol dehydrogenase	1.1.1.1	ADH-1	MF
Adenylate kinase	2.7.4.3	AK-1	TC
Creatine kinase	2.7.3.2	CK-1 CK-2	TC
Esterase	3.1.1.	EST-1 EST-2	TC
Glycerol-3-phosphate dehydrogenase	5.3.1.9	GPD-1 GPD-2	RW
Glucose-6-phosphate isomerase	1.1.1.8	-GPI-1 -GPI-2	RW
Isocitrate dehydrogenase	1.1.1.42	IDH-1	TC
L-lactate dehydrogenase	1.1.1.27	LDH-1 LDH-2	RW
Mannose-6-phosphate isomerase	5.3.1.8	MPI-1	TC
Malate dehydrogenase	1.1.1.37	MDH-1	TC
Peptidases: Glycyl-leucine Leucyl-glycyl-glycine Leucyl-tyrocine	3.4.-	PEP- GL-1 LGG-1 LT-1	RW
6-Phosphogluconate dehydrogenase	1.1.1.44	PGD-1	MF
6-Phosphoglucomutase	2.7.5.1	PGM-1 PGM-2	RW
General proteins	-	PRT-1 PRT-2 PRT-3	PAGE
Superoxide dismutase	1.15.1.1	SOD-1 SOD-2	RW

Table 3.2. Polymorphic loci, alleles resolved (with relative mobilities) and genetic diversity coefficients in three *Cercopithecus aethiops* populations.

Population:		T.V.L	Natal	E. Cape
Locus:	Allele			
MPI-1	100	0.90	0.90	0.72
	87	0.10	0.10	0.28
		h = 18%	h = 18%	h = 40%
PGD-1	147	0.31	0.21	0.11
	100	0.69	0.79	0.89
		h = 42.8%	h = 33.2%	h = 19.6%
PRT-2	114	-	-	0.10
	100	1.00	0.90	0.90
	93	-	0.10	-
			h = 18.0%	h = 18.0%
A:		1.077	1.115	1.115
PPL:		7.69%	11.54%	11.54%
H_{obs}:		2.5%	2.9%	3.3%
H_{exp}:		1.9%	1.7%	2.0%

Table 3.3. Chi-square test for deviation from Hardy-Weinberg equilibrium in three populations.

Population	Locus	Class	Observed frequency	Expected frequency	Chi-square	Degrees of freedom	Level of Significance
T.V.L	MPI-1	AA	0.00	0.01	0.059	1	0.808
		AB	0.20	0.19			
		BB	0.80	0.80			
	PGD-1	AA	0.62	0.47	6.188	1	0.013
		AB	0.15	0.44			
		BB	0.25	0.09			
Natal	MPI-1	AA	0.00	0.01	0.059	1	0.808
		AB	0.20	0.19			
		BB	0.80	0.80			
	PGD-1	AA	0.75	0.62	8.337	1	0.004
		AB	0.08	0.34			
		BB	0.15	0.04			
	PRT-2	AA	0.90	0.80	19.059	1	0.001
		AC	0.00	0.19			
		CC	0.10	0.01			
E. Cape	MPI-1	AA	0.11	0.07	0.503	1	0.478
		AB	0.33	0.42			
		BB	0.55	0.51			
	PGD-1	AA	0.89	0.74	17.067	1	0.001
		AB	0.00	0.20			
		BB	0.11	0.06			
	PRT-2	AA	0.80	0.80	.059	1	0.808
		AB	0.20	0.19			
		BB	0.00	0.01			

Table 3.4. Contingency chi-square analysis at all polymorphic loci.

Locus	No. of alleles	Chi-square	Degrees of freedom	Level of Significance
MPI-1	2	2.993	2	0.224
PGD-1	2	2.423	2	0.298
PRT-2	3	8.143	4	0.086
(Totals)		13.559	8	0.094

Table 3.5. Pair-wise contingency chi-square analysis at all polymorphic loci.

Populations	Locus		
	MPI-1	PGD-1	PRT-2
T.V.L - Natal	0.999	0.424	0.147
T.V.L. – E. Cape	0.158	0.126	0.147
Natal – E. Cape	0.158	0.403	0.135

Table 3.6. Summary of F-statistics at all loci.

Locus	F(IS)	F(IT)	F(ST)
MPI-1	.037	.087	0.052
PGD-1	.751	.761	0.039
PRT-2	.444	.474	0.053
Mean	.436	.462	0.046

Table 3.7. Summary of pair-wise F- statistics and computed gene flow.

Populations	F-statistics & N_{em}			
	F_{ST}	N_{em}	F_{IS}	F_{IT}
T.V.L - Natal	0.015	16.310	0.509	0.516
T.V.L. - E. Cape	0.055	4.300	0.372	0.407
Natal – E. Cape	0.033	7.266	0.451	0.469

Table 3.8. Genetic distances (Nei, 1972) among three populations of vervet monkeys.

Populations	Transvaal	Natal
T.V.L	-	-
Natal	0.001	-
E. Cape	0.003	0.002

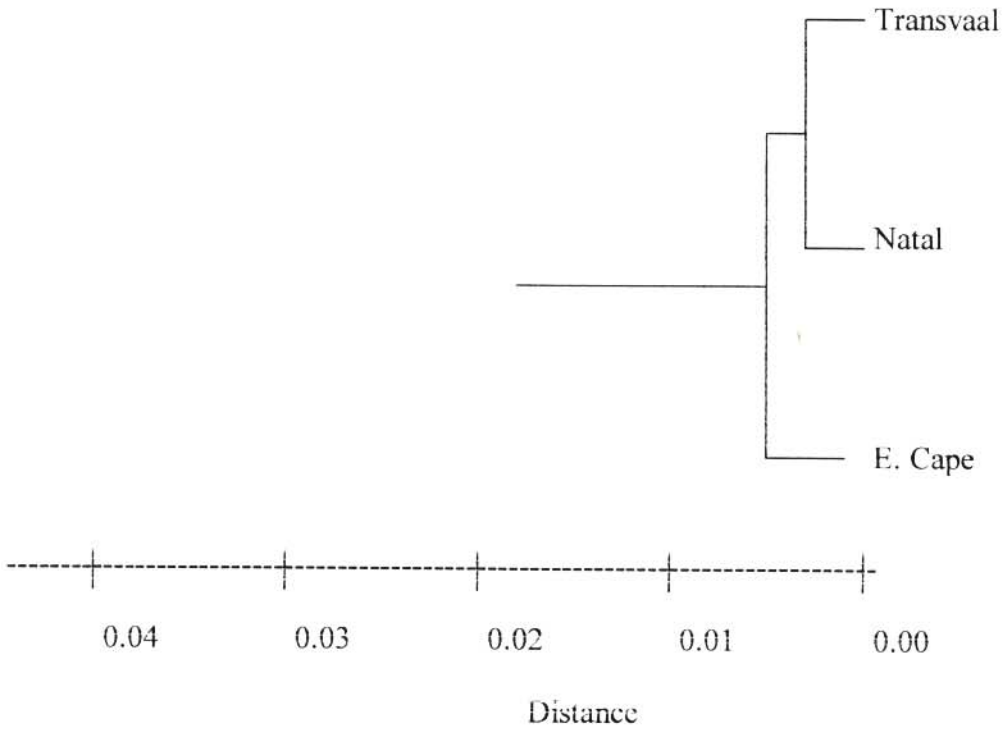

Figure 3.1. Dendogram illustrating relationships among the populations based on genetic distance of Nei, 1972 (Cophenetic correlation = 0.877)

MORPHOLOGICAL ANALYSIS
CHAPTER FOUR

CONTENTS:

4.1. INRODUCTION.

4.1.1. The history of morphological studies.

Morphological differentiation has long been employed to identify taxonomic groups (Sumner, 1924; Williams, 1977; Chambers and Bayless, 1983; and Braun and Mares, 1995). Morphometrics includes the quantification of overall phenotypic similarities of populations by multivariate statistical analysis of measurements of many different characters (Chambers and Bayless, 1983). The ability to apply morphometrics to the skeletal and other remains of preserved organisms from museum collections makes morphometrics applicable and user-friendly to most researchers. Morphological differentiation has also been proven to be a useful tool when coupled with molecular genetic techniques (Patton *et al.*, 1975; Gould and Woordruff, 1978; Schaal and Levin, 1978; Seidel and Lucchino, 1981; Formas, 1991; Formas *et al.*, 1983; Formas *et al.*, 1991; Schneider *et al.*, 1992; Tawfik *et al.*, 1994; Best *et al.*, 1996 and Sugg *et al.*, 1997). Comparisons of morphometric and genetic traits can help researchers to discern how evolutionary forces may act differently on these traits (Sugg *et al.*, 1997).

Several studies in mammals have indicated that there is a relationship between molecular and morphological evolution (Roed, 1987; Scribner *et al.*, 1989; Hartl *et al.*, 1990 and Sugg *et al.*, 1997). Genes interacting at some developmental level control morphological characters, and it is reasonable to assume a relationship between geographic patterns of morphometric and genetic variation (Sugg *et al.*, 1997). It was therefore decided to include a morphological component in the current study to supplement the biochemical genetic study.

Over the last few decades several developments have raised questions within the systematic community as to whether morphometric data should be utilized at all (Felsenstein, 1972, 1985, 1988; Harvey and Pagel 1991; Gittleman and Luk, 1992; Zelditch *et al.*, 1995 and Rohlf, 1998). There had often been controversy between morphological and molecular biology based studies, although this was sometimes brought about by improper project design, flawed philosophy and incorrect methods of data analysis (Crowe, 1988 and Felsenstein, 1988). Morphologists dominated the field of taxonomy during the first century after the publication of Darwin's *Origin of species*. In the search for patterns of true similarity (homology), morphologists employed taxonomic characters such as plumage, bones and other visible aspects of animals. They used the results to erect what then appeared to be a robust classification system. Problems were encountered when there was convergent (analogous) similarity between unrelated organisms and this impacted negatively on morphology based research (Cracraft 1981; Olson, 1981 and Raikow, 1985).

An acceptance of the limitations of morphology was concurrent with the rise of molecular systematics during the last 40 years. Proponents of molecular systematics use techniques such as chromosome banding (Stock and Bunch, 1982), protein electrophoresis (Sibley, 1960; Sibley and Ahlquist, 1972; Barrowclough, 1983; Gutierrez *et al.*, 1983; and Grobler and Matlala, 1998), protein sequencing (Henderson *et al.*, 1981), microcomplement fixation (Jolles *et al.*, 1976 and Prager and Wilson, 1976), DNA restriction mapping (Glaus *et al.*, 1980 and Helm-Bychoski and Wilson 1986), random amplified polymorphic DNA's (Comincini *et al.*, 1996), restriction fragment length

polymorphisms (Gillespie *et al.*, 1995); amplified fragment length polymorphism (Folkertsma *et al.*, 1996) and microsatellites (Herbinger *et al.*, 1995). All these molecular techniques use the body parts and materials, which were often discarded by morphologists (e.g. hair, blood, eye lenses, egg whites, muscles, livers, other internal organs and fecal deposits). These techniques provide solutions to many of the insoluble problems encountered by morphologists. Nevertheless, some morphologists do not appreciate the contribution made by molecular systematics. Instead, they view molecular systematists as misguided biochemists with a solution in search of an application. There had been some counter arguments from molecular systematics such as suggestions that morphological systematists give some characters more weight than others to produce trees of biased topology; choosing between alternative phylogenies primarily on the basis of who had proposed them; and basing their phylogenies on flimsy or equivocal evidence (Crowe, 1988).

It is beyond doubt that both morphology and molecular approaches have some drawbacks. It is recommended that more than one aspect of organisms be studied to get a robust phylogeny (e.g. anatomy, calls, behaviour, proteins and DNA). If all the data sets from all aspects conform, there is high chance of true phylogeny. If the data sets give different trends, there should be a resolution of character conflicts parsimoniously. This implies that, the phylogenetic tree with least and simplest steps will be accepted and used in taxonomy. With this approach morphology and molecular biology will move ahead together to discover the phylogenetic truth (Crowe, 1988).

4.1.2. Factors influencing morphometric studies.

It is often difficult to carry out morphological studies due to the influence of factors such as sex ratio and growth allometry. In some natural populations there are uneven numbers of male and female animals. For example, there are often one or two male vervet monkeys heading a troop of monkeys consisting of numerous females and few young male monkeys (Henzi and Lucas, 1980 and Cheney and Seyfarth, 1983). If sexual dimorphism is pronounced, comparing populations without taking sex ratio into consideration may render the results inaccurate.

There are also cases where the life stages of a particular animal species differ significantly, to such an extent that they may be treated as different species if care is not taken. This is brought about by the process known as growth allometry (Huxley, 1972), i.e. when there are differential growth rates for different parts of the body. If growth allometry is not considered during comparison of populations the results are likely to be inaccurate, especially when one population is dominated by a particular life stage, which is not prevalent in other populations.

4.1.3. Morphometric ratios versus raw data.

In morphology based studies the use of morphometric ratios represents a practical and preferable alternative to use of raw morphological data (Formas *et al.*, 1991; Formas 1993 and Schneider *et al.*, 1992). Reist (1985) pointed out that morphometric ratios are univariate transformations that attempt to separate size and shape variation. When the use

of ratios is coupled with appropriate coding methods and other techniques, it could resolve several inconsistencies in contemporary systematic practice.

4.1.4. Objectives of the morphological comparison of vervet monkey populations.

This component of the present study attempts to:

- identify morphological characteristics and morphometric ratios which can be used in population studies of vervet monkeys;
- identify characteristics that are not influenced by age (growth allometry).
- ascertain if the morphological results coincide with molecular genetic results.

4.2. MATERIALS AND METHODS

A total of 51 vervet monkeys was utilised for morphological analysis. These are the same monkeys described in Chapter 2 and analyzed by biochemical genetic means in Chapter 3. Most published morphometric studies of primates are based solely on paleoanthropology (Rose, 1984; 1988; 1996 and Learmy *et al.*, 1999). The emphasis is on fossils that shed light on the origin of humans and their relationship with other primates. Morphometric studies of living primates are less common. Most species of African primates are listed as endangered in the IUCN red data book and this makes sampling problematic (Lee *et al.*, 1988), which means that there was no work to refer to directly for the choice of morphological characteristics to study. There was no balance between opposite sexes and it was therefore not possible to investigate the influence of sex on morphological analysis. The following sixteen morphological characters, which are

commonly used in morphological studies (DeBlase and Martin, 1981), were thus used: All measurements were taken in centimetres (cm) with exception of weight, which was in kilograms (kg).

- Head length (**HEL**, from the midpoint between the eyes across to the back of the head).
- Head width (**HEW**, from ear to ear).
- Ear length (**EAL**, from the mid-dorsal to the mid-ventral of the pinna).
- Ear width (**EAW**, distance across the centre of the ear).
- Top canine length (**TCL**).
- Top canine diameter (**TCD**).
- Bottom canine length (**BCL**).
- Bottom canine diameter (**BCD**).
- Body length (**BDL**, from the point between the shoulders to the base of the tail).
- Tail length (**TAL**, from the base of the tail to the tip of the tail).
- Hand length (**HAL**, from the tip of the middle finger to the base of the palm).
- Lower arm length (**LWA**, from the wrist to the elbow).
- Upper arm length (**UPA**, from the elbow to shoulder).
- Foot length (**FTL**, from the tip of the middle toe to heel).
- Finger length (**FIL**, length from the middle finger).
- Toe length (**TOL**, length of the middle toe).
- Body mass (kg).

From the 16 morphological characters, nine morphometric ratios were derived for further analysis. The following ratios were used:

- Head width/ head length (**HEW/HEL**).
- Ear width/ head width (**EAW/HEW**).
- Ear width/ ear length (**EAW/ EAL).**
- Top canine diameter/ top canine length (**TCD/ TCL**).
- Bottom canine diameter/ bottom canine length (**BCD/ BDL**).
- Tail length/body length (**TAL/ BDL**).
- Hand length/ lower arm length (**HAL/ LWA**).
- Middle finger length/hand length (**FIL/ HAL**).
- Toe length/ foot length (**TOL/ FTL**).

During the collection of morphological data, the question that arose was:

- are there any significant morphological differences among age groups? This information is of paramount importance as one region might predominantly have a particular age group and during comparison the results are likely to be biased.

The vervet monkeys were therefore categorised into age groups to study the influence of age (growth allometry). A relative age was assigned to each monkey based on comparison with other individuals in the sample whose age was known and this was complemented by information gathered from weight as an indicator of age. Body weight measurement was used for age determination because of its simplicity and also because of lack of other suitable criteria obtainable from live monkeys like measurement of lens

protein, periosteal lines in bone, tooth wear and weight of eye lens (DeBlase and Martin, 1981). However, that was a rough estimate of relative age because monkeys display indeterminate growth (the absence of well-defined stages of growth and of fixed sizes of the adults). The following age groups were described wherein monkeys were categorised:

- Adult (large and potentially breeding monkeys. Weight about 4.5 kg and above).
- Sub-adult (smaller than adults, but otherwise similar to adults. Weight between 3 and 4.4 kg).
- Juvenile (smaller than the sub-adults. Weight between 1.5 and 2.9 kg).

Analysis of morphometric results proceeded as follows. Firstly, raw data from all three populations were pooled before calculation of nine morphometric ratios, to obtain overall results for each of the three age categories (adult, sub-adult and juvenile) and thus determine the influence of age. From these results, any age group that leads to inconsistent results could be excluded from further analysis. Results for the remaining groups could then be used for comparison between geographical regions.

The computer program Statistical Products and Service Solutions (SPSS-statistical package, 1997 version) was used to analyse morphometric data. Significance of variance among geographical and age groups were calculated using one way ANOVA and Bonferroni's multiple comparison.

4.3. RESULTS.

It is of the utmost importance to ascertain if there are significant differences among age groups within a population before any possible comparisons between populations are made (Huxley, 1972, Blackstone, 1987). For this reason, data from all populations were first pooled and then divided into age-classes (Table 4.1), to test for significance of differences among age groups. The mean values for nine morphometric ratios for three pooled age-classes are presented in Table 4.2. Significant differences ($p<0.001$ to $p<0.009$) among age-classes were observed for five ratios (Table 4.2 and figure 4.1). Multiple comparisons (Table 4.3) showed that significant ($p<0.01$ to $p<0.02$) differences between juveniles and older age-classes of monkeys occurred for five ratios. Differences between adults and sub-adults were mostly insignificant ($p<0.149$ to $p<0.999$), with only one instance of significance ($p<0.001$, for ear width / ear length). It was thus decided to exclude the latter ratio and all data for juveniles from further calculations, and to pool data for adults and sub-adults in each geographical region (Table 4.4). Mean values for the remaining eight morphometric ratios for each of the three geographical regions studied are presented in Table 4.5 and figure 4.2. None of the differences among mean values of different geographic regions was found to be significant ($p<0.099$ to $p<0.706$). It was therefore not necessary to proceed with Bonferroni's multiple comparison of mean differences as previously done among age groups.

4.4. DISCUSSION

4.4.1. Suitability of characters for morphological comparison.

Multiple comparisons of mean differences showed that morphometric differences were more pronounced between juvenile and adult categories than between adults and sub-adults (Table 4.2). This close relationship of sub-adults to adults reflects the process of sub-adults preparing physiologically and physically for adulthood. The morphological differences among age groups can be attributed to the concept of growth allometry (differential growth rate of different parts of the body). Relative growth occurs when different "polyclones", which make up different tissues grow at different cell division rates (Blackstone, 1987). Huxley (1972) reported three types of allometric relationships:

- Isometric relationship (the body part and the body as a whole always remain in the same proportion),
- Negative allometry (the body part becomes proportionately smaller as the body grows), and
- Positive allometry (the body part increases in size faster than the total body).

The morphometric differences among vervet monkey population age groups have shown that some characters have negative allometry and others have positive allometry. In the ratio head width / head length, head length seems to decrease (mean difference among age groups with $p<0.009$) in relation to head width as the monkeys grow, indicating negative allometry. In the ratio ear width / head width, head width seems to increase faster than ear width even though it is insignificant (mean difference among age groups with $p<0.078$), suggesting some positive allometry. For tail length / body length, tail

length and body length increase linearly as the monkeys grow (mean difference among age groups with $p<0.115$), representing isometric relationship. If different allometric relationships exist within a sample, geographic variation in shape is likely to be biased. This warrants a further study on allometry in the future because comparison of samples ideally should be in terms of shape variates free from magnitude effects such as size (brought about by different life stages).

Body weight was utilised to divide monkeys into age groups (juvenile, sub-adult and adult). However, these monkeys originated from different geographical regions with different environmental circumstances and climate. If an animal of known age live under captive conditions, the morphological changes that occur during development may be different to the changes experienced by individuals living in the wild and thus the results obtained from captive animals may not be entirely accurate for purposes of aging. Lee *et al.* (1986) reported that vervet monkeys around lodges, settlements and garbage disposal sites do not follow the same strictly seasonal pattern of reproduction as observed in their wild counterparts. This altered environmental condition may have an impact on the morphology of the vervet monkeys. Wild vervet monkeys are usually slim and active. These adaptations confer the wild vervet monkeys with the ability to move fast and avoid being caught by predators (Markowitz and Spinelli, 1986 and Harris 1988). In contrast, the captive vervet monkeys are less active, confined to the enclosures, fed regularly and often overweight as compared to their wild counterparts.

4.4.2. Geographical variation.

The morphological data obtained during the current study do not provide any motivation for the continued separation of vervet monkeys originating from the former Transvaal, KwaZulu-Natal and the Eastern Cape regions of South Africa. One-way ANOVA of mean ratios between and among groups indicated that there are no profound morphometric differences among South African vervet monkey populations from different geographical regions (Table 4.5). This suggests that, morphologically, vervet monkeys from a wide distribution area across South Africa are monotypic.

Geographical patterns of variances are expected to be similar for morphometric and genic traits under some conditions, but not others (Sugg *et al.*, 1997). Trends observed from morphological results during the present study do not support the conclusion based on genetic analysis. There are several ways to account for this. The sample sizes used during the morphological study were relatively small. Also, males and females were not separated, since this would have resulted in an unacceptable further stretching of already small sample size. The pooling of sexes may have introduced inconsistencies into the morphological analysis. Nevertheless, the current study included a wide range of body parameters, and failure to detect any significant morphological differences between conspecific populations may not be the result of sampling error. In situations where environments are heterogeneous and selection pressures can differentiate among groups, patterns for genic and morphometric traits may not be congruent (Sugg *et al.*, 1997). It is possible that genetic divergence between geographically distant vervet monkeys have not resulted in significant morphological differences.

4.5. APPENDIX

Table 4.1. Morphological data for 16 body characters: geographical regions pooled into three age groups.

Character:	Age category:	Mean value:
Weight	Juvenile Sub-adults Adults	2.197±0.443 3.342±0.288 5.500±0.267
Head length	Juvenile Sub-adults Adults	81.017±10.493 86.000±13.810 88.750±11.068
Head width	Juvenile Sub-adults Adults	60.357±4.653 67.750±4.393 76.286±5.122
Ear length	Juvenile Sub-adults Adults	37.194±3.060 42.400±2.675 44.500±6.949
Ear width	Juvenile Sub-adults Adults	27.742±2.462 28.300±4.498 31.875±4.549
Top canine length	Juvenile Sub-adults Adults	6.833±3.373 9.800±0.919 21.125±3.980
Top canine diameter	Juvenile Sub-adults Adults	4.250±1.215 4.800±0.587 7.875±1.576
Bottom canine length	Juvenile Sub-adults Adults	5.667±2.229 8.450±1.571 12.750±2.053
Bottom canine diameter	Juvenile Sub-adults Adults	3.250±0.622 3.650±0.818 5.000±1.069
Body length	Juvenile Sub-adults Adults	285.194±27.163 338.333±24.340 392.500±27.646
Tail length	Juvenile Sub-adults Adults	477.633±73.944 579.250±43.447 585.000±36.742

Table 4.1 Continued...

Character:	Age category:	Mean value:
Hand length	Juvenile Sub-adults Adults	74.586±5.349 84.333±5.614 92.500±3.928
Lower arm length	Juvenile Sub-adults Adults	112.231±11.187 145.125±13.580 157.500±12.145
Foot length	Juvenile Sub-adults Adults	116.897±18.345 126.091±8.324 132.429±18.311
Finger length	Juvenile Sub-adults Adults	29.821±2.092 33.500±3.826 37.429±3.102
Toe length	Juvenile Sub-adults Adults	30.926±3.731 37.167±4.086 38.429±4.353

Table 4.2. Mean values and significance of differences for nine morphometric ratios calculated for three age groups (pooled geographical regions) of vervet monkeys. Differences considered significant at the 0.05 level are indicated with the symbol *.

Ratio	Age Category	Mean	Significance
Head width / head length	Juvenile Sub-adult Adult	0.747±0.084 0.802±0.110 0.865±0.081	p<0.009*
Ear width / head width	Juvenile Sub-adult Adult	0.465±0.060 0.423±0.042 0.419±0.060	p<0.078
Ear width / ear length	Juvenile Sub-adult Adult	0.059±0.012 0.079±0.091 0.128±0.029	p<0.001*
Top canine diameter / top canine length	Juvenile Sub-adult Adult	0.705±0.216 0.492±0.056 0.381±0.087	p<0.001*
Bottom canine diameter / bottom canine length	Juvenile Sub-adult Adult	0.632±0.184 0.445±0.129 0.399±0.099	p<0.003*
Tail length / body length	Juvenile Sub-adult Adult	1.687±0.275 1.718±0.149 1.477±0.161	p<0.115
Hand length / lower arm length	Juvenile Sub-adult Adult	0.673±0.060 0.588±0.058 0.588±0.038	p<0.001*
Finger length / hand length	Juvenile Sub-adult Adult	0.402±0.028 0.402±0.072 0.408±0.022	p<0.939
Toe length / foot length	Juvenile Sub-adult Adult	0.269±0.043 0.297±0.050 0.295±0.049	p<0.164

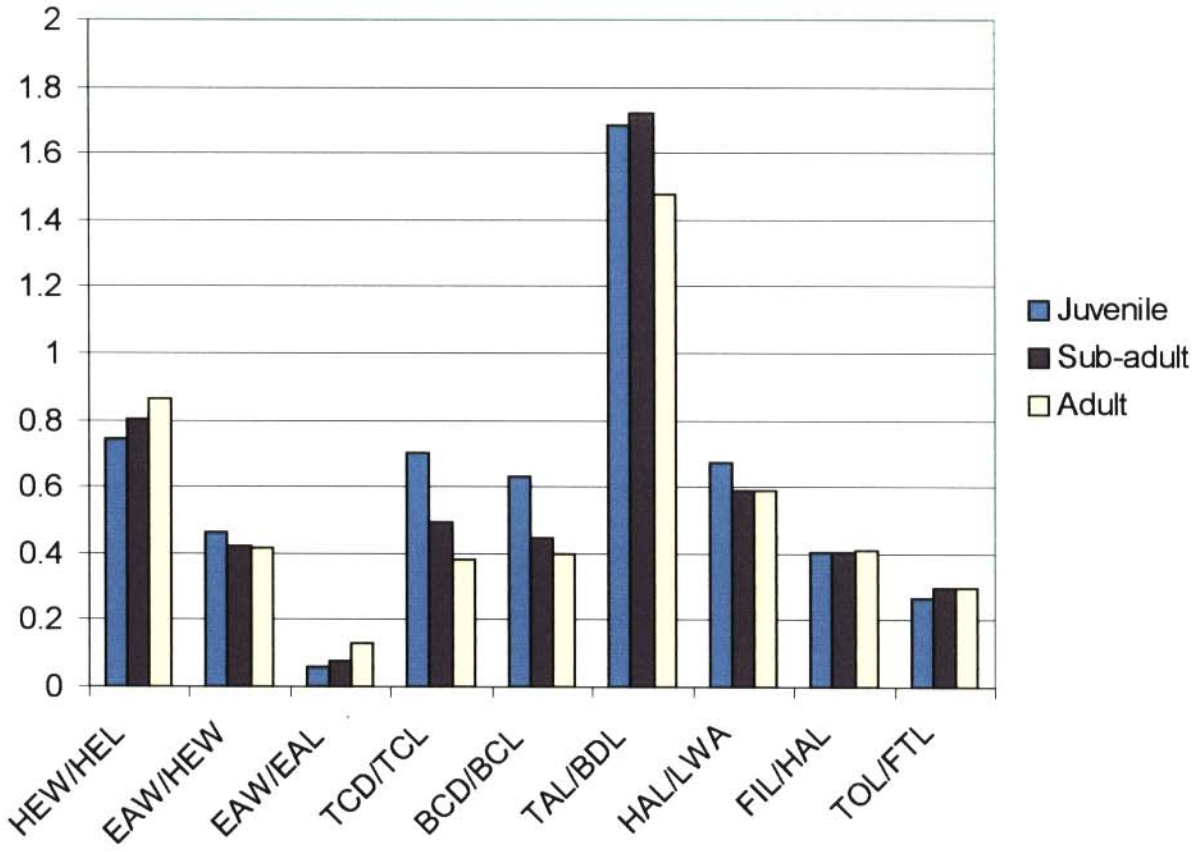

Figure 4.1. Means of morphometric ratios of three age groups of vervet monkeys.

Table 4.3. Multiple comparisons of morphometric ratios among age groups (pooled for geographical regions). Differences considered significant at the 0.05 level are indicated with the symbol * and # indicates the only significant difference between sub-adult and adult.

Morphometric ratio:	Age categories:		Significance:
Head width / head length	Juvenile	- Sub-adult	p<0.259
		- Adult	p<0.011*
	Sub-adult	- Adult	p<0.457
Ear width / head width	Juvenile	- Sub-adult	p<0.199
		- Adult	p<0.254
	Sub-adult	- Adult	p<0.999
Ear width / ear length	Juvenile	- Sub-adult	p<0.003*
		- Adult	p<0.001*
	Sub-adult	- Adult	p<0.001#
Top canine diameter / top canine length	Juvenile	- Sub-adult	p<0.007*
		- Adult	p<0.001*
	Sub-adult	- Adult	p<0.378
Bottom canine diameter / bottom canine length	Juvenile	- Sub-adult	p<0.020*
		- Adult	p<0.006*
	Sub-adult	- Adult	p<0.999
Tail length / body length	Juvenile	- Sub-adult	p<0.999
		- Adult	p<0.168
	Sub-adult	- Adult	p<0.149
Hand length / lower arm length	Juvenile	- Sub-adult	p<0.002*
		- Adult	p<0.008*
	Sub-adult	- Adult	p<0.999
Finger length / hand length	Juvenile	- Sub-adult	p<0.999
		- Adult	p<0.999
	Sub-adult	- Adult	p<0.999
Toe length / foot length	Juvenile	- Sub-adult	p<0.283
		- Adult	p<0.589
	Sub-adult	- Adult	p<0.999

Table 4.4. Morphometric data by geographical region (adults and sub-adults pooled).

Character:	Region:	Mean: (Adult + Sub-adult)
Head length	**Transvaal**	84.222±5.995
	KwaZulu-Natal	91.667±17.259
	Eastern Cape	86.800±15.928
Head width	**Transvaal**	72.250±5.676
	KwaZulu-Natal	70.833±8.159
	Eastern Cape	68.800±4.919
Ear width	**Transvaal**	30.444±4.927
	KwaZulu-Natal	30.000±4.561
	Eastern Cape	28.000±6.000
Top canine length	**Transvaal**	17.889±6.717
	KwaZulu-Natal	13.200±5.805
	Eastern Cape	10.000±0.817
Top canine diameter	**Transvaal**	6.667±1.677
	KwaZulu-Natal	6.500±2.598
	Eastern Cape	4.625±0.479
Bottom canine length	**Transvaal**	11.056±1.377
	KwaZulu-Natal	10.600±1.949
	Eastern Cape	8.500±1.732
Bottom canine diameter	**Transvaal**	4.889±1.167
	KwaZulu-Natal	3.900±0.742
	Eastern Cape	3.250±0.500
Body length	**Transvaal**	381.111±38.225
	KwaZulu-Natal	337.500±27.523
	Eastern Cape	349.000±26.552
Tail length	**Transvaal**	578.571±43.270
	KwaZulu-Natal	590.833±34.701
	Eastern Cape	573.200±48.515
Hand length	**Transvaal**	89.444±6.307
	KwaZulu-Natal	88.500±4.324
	Eastern Cape	83.200±7.596
Lower arm length	**Transvaal**	153.333±17.224
	KwaZulu-Natal	148.750±12.500
	Eastern Cape	147.750±13.048
Foot length	**Transvaal**	128.375±16.826
	KwaZulu-Natal	133.000±8.602
	Eastern Cape	124.400±10.164
Finger length	**Transvaal**	36.250±3.240
	KwaZulu-Natal	34.167±3.817
	Eastern Cape	33.800±5.404
Toe length	**Transvaal**	38.250±3.770
	KwaZulu-Natal	36.333±2.422
	Eastern Cape	38.200±6.340

Table 4.5. Mean values and significance of differences for eight morphometric ratios calculated for vervet monkeys (sub-adults and adults, pooled) from three South African regions.

Morphometric ratio:	Region:	Mean value:	Significance:
Head width / head length	T.V.L Natal E. Cape Total	0.863±0.019 0.788±0.126 0.811±0.133 0.826±0.103	P<0.402
Ear width / head width	T.V.L Natal E. Cape Total	0.420±0.062 0.424±0.053 0.421±0.077 0.422±0.057	P<0.290
Top canine diameter / top canine length	T.V.L Natal E. Cape Total	0.399±0.098 0.501±0.038 0.466±0.075 0.442±0.089	P<0.099
Bottom canine diameter / bottom canine length	T.V.L Natal E. Cape Total	0.471±0.145 0.370±0.055 0.389±0.061 0.425±0.116	P<0.248
Tail length / body length	T.V.L Natal E. Cape Total	1.528±0.204 1.756±0.114 1.649±0.176 1.638±0.189	P<0.087
Hand length / lower arm length	T.V.L Natal E. Cape Total	0.586±0.032 0.615±0.048 0.564±0.068 0.588±0.049	P<0.368
Finger length / hand length	T.V.L Natal E. Cape Total	0.410±0.028 0.387±0.045 0.414±0.104 0.404±0.058	P<0.706
Toe length / foot length	T.V.L Natal E. Cape Total	0.302±0.042 0.273±0.025 0.311±0.072 0.296±0.048	P<0.443

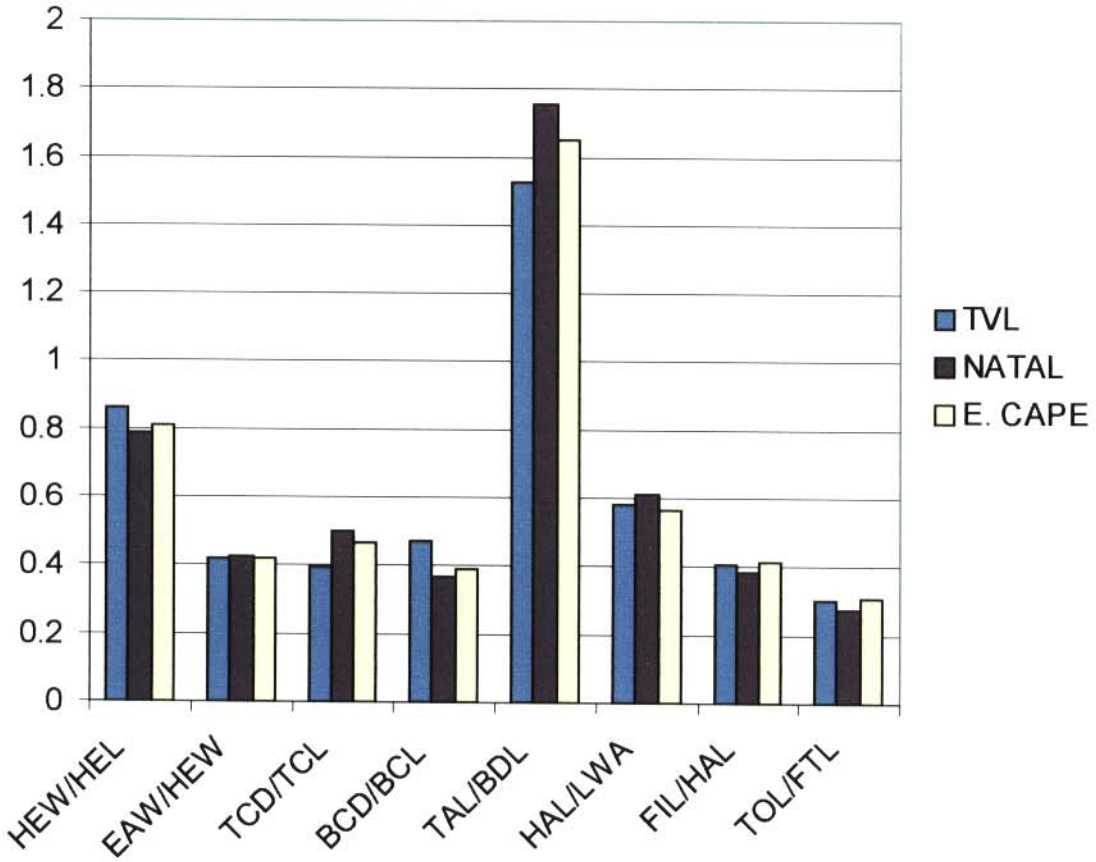

Figure 4.2. Means of morphometric ratios of geographical groups.

CONCLUSION & RECOMMENDATIONS CHAPTER FIVE

The primary aim of this study was **"to establish a database on the distribution of biochemical genetic variability in South African vervet monkey populations; and then use this information in order to find out whether vervet monkeys originating from different regions can be mixed or not"**.

The results of the biochemical genetic study do not provide an unambiguous answer to this research question. From a statistical viewpoint, the vervet monkey populations from different geographical regions are separated by relatively insignificant genetic distances (0.001-0.003). This trend is also supported by the overall fixation index value of 0.046. This implies that vervet monkeys from the former Transvaal, KwaZulu-Natal and the Eastern Cape can be allowed to interbreed because more genetic variation resides within populations than among populations. Genetic divergence among these populations is not significant and will probably be nullified following the resumption of inter-population gene flow.

However, a more detailed analysis of results shows that the vervet monkey populations from KwaZulu-Natal and the Eastern Cape each had unique alleles at the **PTR-2** locus, both of which are not present in the former Transvaal vervet monkey population. These alternate alleles at **PRT-2** favour the option of placing a moratorium on mixing, as it is not yet understood whether the alternative alleles are taxonomically significant or have adaptive implications. If these rare alleles have the adaptive implications then it would be prudent not to allow interbreeding.

Tentatively, the recommendation is that interbreeding of populations at the Riverside Rehabilitation Center, originating from different geographical regions, should not be allowed. However, the present results are based solely on allozyme electrophoresis. It is therefore advisable to follow up this study at the earliest possible opportunity with a study incorporating additional molecular genetic markers, such as microsatellites and RAPDs. It would also be advisable to include data from truly free-ranging vervet monkey populations from the relevant regions in any future study.

The release of rehabilitated monkeys back into their geographic regions of origin can however continue and this should in fact be encouraged, where the origin of vervet monkeys is well known.

Although the present study revealed relatively low levels of genetic diversity, with average heterozygosity values of 2.5-3.3%, it cannot be concluded that the vervet monkey populations suffer from inbreeding depression. Lande (1988) has warned researchers against the assumption that low levels of electrophoretically detectable variation in enzymes necessarily mean that a population lacks heritable variation in quantitative traits or is suffering from inbreeding. Low levels of genetic diversity may also be attributed to historical events rather than recent management decisions; or it could be a species-specific characteristic.

Since molecular based methods are not always a practical proposition for conservation managers at ground level, it was also an aim of this study "**to ascertain to what extent morphological information can supplement biochemical genetic information**" in identifying geographically distinct variants of the vervet monkey.

The results of the genetic and morphological components of this study suggest that there is not absolute congruence between molecular and morphological evolution. Morphological results suggest that vervet monkeys from a wide distribution area across South Africa are monotypic, in contrast to the genetic results that revealed rare alleles found at the locus **PRT-2**. The morphological study did however identify suitable traits, free from the influence of growth allometry that can be used for inter-population comparisons in vervet monkeys.

In conclusion, this study has revealed new data on allozyme variability in a species where such information was previously unavailable. Results show that genetic diversity in

vervet monkeys is comparable to levels in related species, although heterozygosity leans towards the lower end of the spectrum. Morphologically, vervet monkeys from various regions of South Africa appear to be monotypic. This conclusion is supported by a statistical analysis of biochemical genetic data. Nevertheless, there are rare allelic differences between regional populations that suggest that vervet monkeys from the former Transvaal, KwaZulu-Natal and Eastern Cape should, for the immediate future, not be kept in the same enclosures or mixed during release back into the wild.

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