Allozyme variation in African elephant (*Loxodonta africana*) from the Kruger National Park, South Africa

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Genetic variation within and differentiation between juvenile and adult elephants (*Loxodonta africana*) culled during April 1992 and May 1993 were compared to determine the effect of culling on successive generations. Gene products of 25 protein-coding loci were examined by horizontal starch gel-electrophoresis as well as polyacrylamide gel-electrophoresis. Eighteen protein-coding loci (72%) in 1992 and 19 (76%) in 1993 displayed monomorphic gel-banding patterns whereas only seven in 1992 and six in 1993 were polymorphic. Average heterozygosity values for adults, juveniles and the total population in 1992 were 0.058, 0.024 and 0.047 respectively. Lower values (0.037, 0.021 and 0.033 respectively) were obtained for different family groups in the present study, and statistically significant ($p<0.05$) similarities were found between allele distributions at four of the eight polymorphic loci of the two elephant populations. This study confirmed the result of the previous study and new information is presented on variation at an additional locus. The data will be valuable to investigate new means of controlling elephant populations effectively without losing genetic variation.

Genetiese variasie binne, en differensiasie tussen, olifante (*Loxodonta africana*) van die uitdunningsprogramme gedurende April 1992 en Mei 1993 is vergelyk om die effek van uitdunning op opeenvolgende generasies te bepaal. Geenprodukte van 25 proteïen gekodeerde lokusse is ondersoek deur gebruik te maak van horisontale stysel-jel-elektroforese asook poliakrielamied-jel-elektroforese. Agtien proteïen gekodeerde lokusse (72%) in 1992 en 19 (76%) in 1993 het monomorfiese jel-bandpatrone vertoon terwyl slegs sewe in 1992 en ses in 1993 polimorfies was. Gemiddelde heterosigositeitwaardes vir volwassenes, onvolwassenes en die totale bevolking in 1992 was onderskeidelik 0.058, 0.024, 0.047. Laer waardes (0.037, 0.021 en 0.033 onderskeidelik) is bepaal vir 'n verskillende familiegroep in die huidige studie en statisties beduidende ($p<0.05$) ooreenkomste tussen die alleelverspreidings by vier van die agt polimorfiese lokusse van die twee olifantbevolkings is gevind. Die huidige studie bevestig die resultate van die vorige studie en variasie by 'n addisionele lokus word verskaf. Die data sal waardevolle inligting verskaf by die ondersoek van nuwe metodes om die getalle van olifantbevolkings te beheer sonder om waardevolle genetiese variasie te verloor.

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Introduction

Elephants have been successfully conserved and managed in the Kruger National Park (KNP) since 1898. The elephant population has increased at 5–7% per annum to levels where these excessive numbers threaten the integrity and biological diversity of their habitat. Surplus elephants have been culled annually from 1968–1994 to limit the numbers to 7000–7500 animals (Hall-Martin 1990). It

is, therefore, possible that inbreeding has occurred because the elephant population was derived from 10 elephants in 1905 (Stevenson-Hamilton, 1947), and there are presently more than 8000 elephants in the KNP. It was necessary to determine the amount of genetic variation in the KNP elephant population to determine the extent of inbreeding.

The genetic variation of elephants in the central part of the KNP was previously determined (Coetzee *et al.* 1993) and it was found that there was a decline in genetic variation from one generation to the next. This trend was not observed in African buffalo (*Syncerus caffer*) from the KNP (Grobler & Van der Bank, 1996). However, the previous study indicated that the elephants of the KNP still possess a healthy amount of heterozygosity, but that there is a reduction in heterozygosity from adults (5.8%) to juveniles (2.4%). This reduction in genetic variation could mean that future generations would be less adaptable to environmental changes and that these generations could be more susceptible to negative influence. These results could have been susceptible to sample error. The aim of this study is to compare the results reported by Coetzee *et al.* (1993) to those of a different family group from the northern part of the KNP.

Materials and Methods

Elephants at Mooiplaas (23°33'13"S, 31°26'49"E) in the northern part of the KNP were sampled during the penultimate culling programme in 1993 for this study. The results are compared to those of elephants from the central part of the KNP (23°57'S, 31°37'E; Coetzee *et al.*, 1993), which were culled during April 1992 (Figure 1). Whole (heparinised) blood samples were taken from 68 elephants which included adults, sub-adults and calves. Forty-six heart and liver samples were collected from adults and sub-adults. Some of the calves were not culled but captured for translocation. Thirty kidney and 32 muscle samples were also collected. Tissue samples were stored in liquid nitrogen and transported to the laboratory where they were stored at –20°C. No skin, eye and testis samples were collected for this study as these samples did not present additional results during electrophoresis (Coetzee *et al.* 1993).

Thirteen percent starch gels were used to analyse samples, using electrophoretic procedures, buffers, methods of interpretation of gel patterns and nomenclature described in Van der Bank (1993). Statistical analysis of allozyme data was done with BIOSYS-1 (Swofford & Selander 1981). Adults and juveniles (sub-adults and calves) were separately analysed for Hardy-Weinberg equilibrium of genotypes. Deviations of allele classes from expected Hardy-Weinberg equilibrium from observed genotypic frequencies were determined using the Chi-square (χ^2) goodness of fit test. Genetic variability was analysed using average heterozygosity (H), proportion of polymorphic loci (P) and mean number of alleles per locus (A) values. We used GENEPOP (Raymond & Rousette 1997) for exact tests of population differentiation, and DISPAN (Ota 1993) to show relationships between the elephants studied.

Two computer simulation programmes (DRFTMUSL and SELECTIN) were used to estimate the duration of genetic variability and gene transfer (Gilpin, 1987). DRFTMUSL utilises the population size, mutation rate and selection coefficients as parameters to simulate the combined effect of random genetic drift, mutation and natural selection. The selection coefficients as well as the initial allele frequencies of the B-allele are specified in SELECTIN. Selection coefficients ($\underline{s}$) were calculated using $\underline{s} = 1 - \underline{w}$, where $\underline{w}$ depicts Darwinian fitness or relative reproductive efficiency (Ayala 1982; Price & Boag 1987).

Results and Discussion

Twenty-five protein-coding loci were resolved by starch gel-electrophoresis and provided interpret-

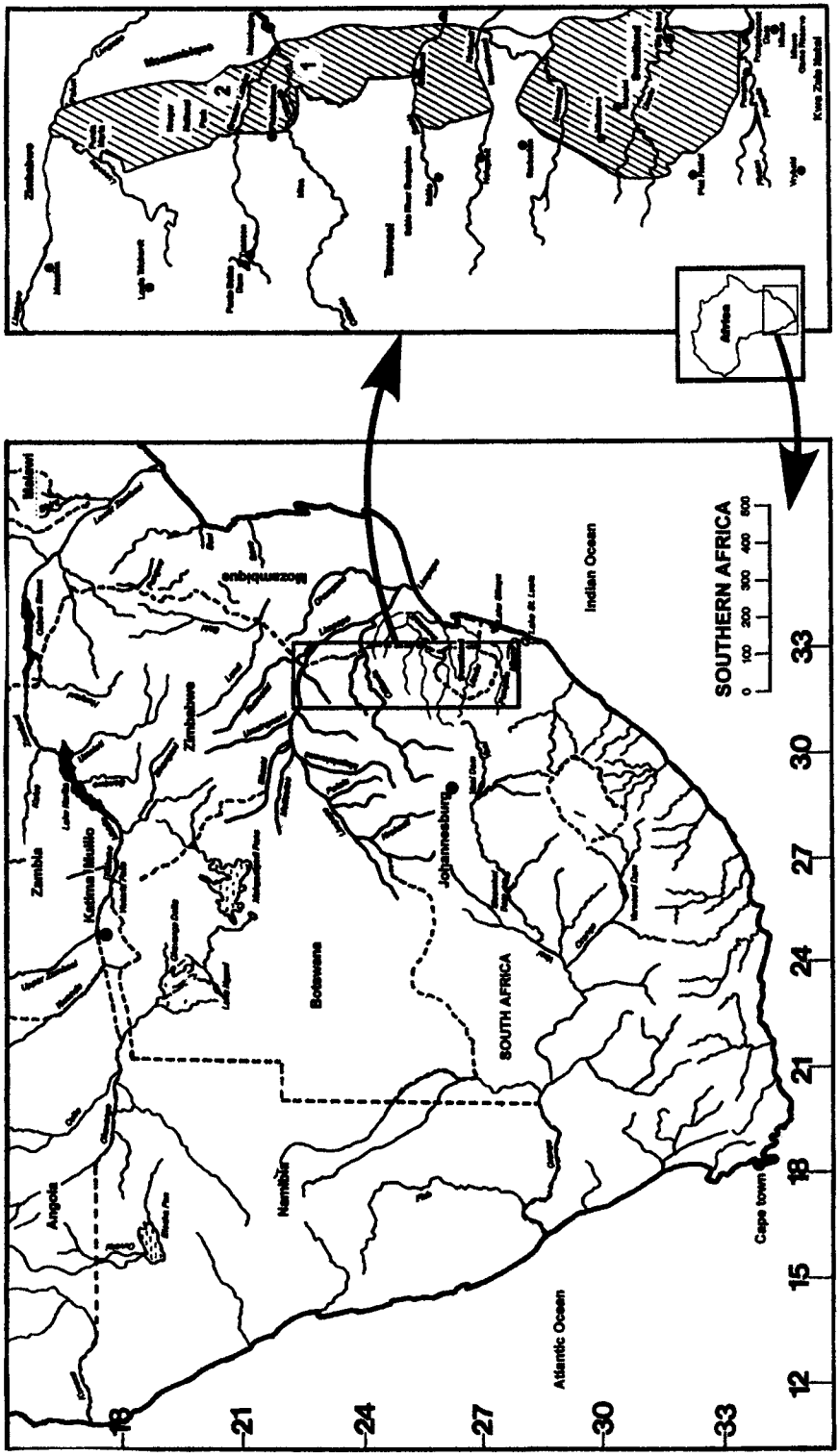

Figure 1 Map showing sampling localities (1 = 1992 and 2 = 1993 culling sites).

able results. Locus abbreviations, enzyme commission numbers, tissue and buffers giving the best results are listed in Table 1. Nineteen of the loci (76%) displayed monomorphic gel banding patterns and six loci (ALB, CK-1, EST-2, LDH-1, LDH-2 and SDH) displayed polymorphic gel banding patterns (Table 2). The preliminary study by Coetzee *et al.* (1993) reported polymorphic gel banding patterns at seven loci (ALB, CK-1, EST-2, IDH, LDH-1, LDH-2 and MDH-1). Allele frequencies for polymorphic loci, χ^2 values, and sample size are presented in Table 2.

Polymorphism was found in blood samples collected by Drysdale & Florkiewics (1988) from Savannah elephants (*L. africana africana*), forest elephants (*L. africana cyclotis*) and Asian elephants (*Elephas maximus*). The polymorphic loci were LDH, PGM, SOD and a general plasma protein. Osterhoff *et al.* (1974) also found genetic differentiation in serum transferrin, albumin and esterase in blood samples of elephants in the KNP and Brown *et al.* (1978) found no genetic variation in a single Ugandan elephant population. To date, these were the only electrophoretic studies on elephants except for the preliminary study by Coetzee *et al.* (1993).

Polymorphism was observed in two of the 47 blood samples examined for albumin in the present study. These individuals also displayed the alternative (B) allele reported in the study by Coetzee *et al.* (1993). The allele classes at this locus did not deviate from expected Hardy-Weinberg proportions and all of the juveniles were monomorphic for this enzyme (Table 2).

The monomeric CK-1 enzyme coding locus displayed four double banded heterozygotes in heart and liver tissue in adults and were monomorphic for all of the juveniles, which compares well with previous results (Coetzee *et al.* 1993). Significant ($p<0.05$) deviations of genotypic frequencies occurred for elephants in both studies (Table 2).

Esterase-2 was monomorphic in juvenile elephants whereas EST-1 and EST-3 were monomorphic for both adults and juveniles. Esterase-2 was polymorphic in two liver samples in adult elephants and the genotypes at EST-2 deviated from expected Hardy-Weinberg proportions (Table 2). These results also compare well with previous results (Coetzee *et al.* 1993).

Table 1 Protein, locus abbreviations, enzyme commission number (EC No.), buffers and tissues giving the best results

Protein	Locus	EC No.	Buffer	Tissue
Albumin	*ALB		PAGE	Blood
Creatine-kinase	CK-*1 -4	2.7.3.2	TC	Heart/Liver
Esterase	EST-1,*2,3	3.1.1	RW	Liver
Glucose-6-phosphate isomerase	GPI- 1,2	3.5.1.9	TC	Heart/Liver
Haemoglobin	HB		MF	Blood
Isocitrate dehydrogenase	IDH	1.1.1.42	RW	Kidney
Lactate dehydrogenase	LDH-*1,*2,3	1.1.1.27	MF	Blood
Peptidase:				
Substrate: leosine-tyrosine	LT-1,2	3.4.1.1	RW	Heart/Liver
Malate dehydrogenase	MDH-1,2	1.1.1.37	TC	Muscle
Manose-6-phosphate isomerase	MPI-1,2	5.3.1.8	MF	Blood
Superoxide dismitase	SOD	1.15.1.1	RW	Liver
Sorbitol dehydrogenase	*SDH	1.1.1.14	RW	Kidney
General protein	PROT-I-3		MF	Blood

* polymorphic loci

Table 2 Sample sizes, allelic frequencies at polymorphic loci and χ^2 values for adults, juveniles and for the total elephant populations sampled in 1992 and 1993

Locus:		Allele:	Adults	Juveniles	Total('93)	Total('92)
ALB	2N		94	42	136	122
		A	0.957	1.000	0.971	0.984
		B	0.043		0.029	0.016
	χ^2		47.00*		68.00*	121.008*
CK- 1	2N		64	28	96	60
		A	0.875	1.000	0.917	0.700
		B	0.125		0.083	0.300
	χ^2		32.00*		48.00*	31.455*
EST-2	2N		64	28	96	60
		A	0.938	1.000	0.957	0.967
		B	0.062		0.043	0.033
	χ^2		32.00*		46.00*	59.018*
IDH-1	2N				60	86
		A	1.000	1.000	1.000	0.802
		B				0.198
	χ^2					0.342
LDH-1	2N		94	42	136	122
		A	0.851	0.952	0.875	0.918
		B	0.149	0.048	0.125	0.082
	χ^2		47.00*	21.00*	64.00*	67.267*
LDH-2	2N		94	42	136	118
		A	0.915	0.905	0.906	0.951
		B	0.085	0.095	0.094	0.049
	χ^2		47.00*	21.00*	64.00*	72.626*
MDH-1	2N				60	86
		A	1.000	1.000	1.000	0.953
		B				0.047
	χ^2					56.691*
SDH	2N		40	20	60	90
		A	0.950	0.850	0.917	1.000
		B	0.050	0.150	0.083	
	χ^2		0.050	0.311	0.248	

*Significant ($p<0.05$) deviation of allele frequency from expected Hardy-Weinberg proportions.

No polymorphism was found for IDH in liver tissue, as was found in the previous study (Coetzee *et al.* 1993). No other results for IDH could be found in the literature for comparison.

Lactate dehydrogenase-1 displayed polymorphism in seven adults and one juvenile. The genotypes at LDH-1 deviated from expected Hardy-Weinberg proportions (Table 2) which are similar to results by Coetzee *et al.* (1993) and Drysdale & Florkiewics (1988) for blood samples from Indian and African elephants. Lactate dehydrogenase-2 showed polymorphism in blood samples of four adults and two juveniles. Allele classes also deviated from expected Hardy-Weinberg proportions at this locus (Table 2). Osterhoff *et al.* (1974) found no polymorphism for this tetrameric enzyme.

Polymorphism was not found in the present study for MDH in kidney samples as was obtained in the study by Coetzee *et al.* (1993). No other results for MDH of elephants could be found in the literature.

Sorbitol dehydrogenase displayed polymorphism in two adults and three juveniles in kidney tissue. No polymorphism was found at this locus by Coetzee *et al.* (1993) and no other results could be found in the literature for SDH of elephants. Allele distributions did not deviate from expected Hardy-Weinberg proportions at this locus (Table 2).

Deviations of genotypes from expected Hardy-Weinberg equilibrium occurred at five (ALB, CH-1, EST-2, LDH-1 and LDH-2) of the six enzymes (Table 2). There are various factors that could cause deviations from expected Hardy-Weinberg proportions such as sampling error, recurrent bottlenecks, sample size and the Walhund effect. These factors were discussed by Coetzee *et al.* (1993) and will not be repeated.

Average heterozygosity values for adults, juveniles and the total population were 3.7 (±0.015), 2.1 (±0.012) and 3.3% (±0.013) respectively. Therefore, our results show that there is a decrease in H values from adults (3.7%) to juveniles (2.1%). The H value for all individuals (3.3%) in the present study is also lower than the value (4.7%) reported by Coetzee *et al.* (1993). This trend is also reflected by the A and P values (A: 1.28 for 1992 and 1.24 for 1993 and P: 28% and 24% for 1992 and 1993 respectively). The average H value for these two populations is 4%, and these results are closer to the average estimate of H (approximately 3%) for large mammals (Nevo *et al.* 1984) and for buffalo from the KNP (Grobler & Van der Bank 1996), 4% for blue wildebeest (*Connochaetes taurinus*) (Grobler & Van der Bank 1993), sable antelope (*Hippotragus niger*) (Grobler & Van der Bank 1994a) and 4.6% for impala (*Aepyceros melampus*) (Grobler & Van der Bank 1994b). The high H value of 8.9% obtained by Drysdale & Florkiewics (1988) for eight African elephants should not be used as an indication of high heterozygosity owing to the small sample size and the fact that their samples did not represent a natural population.

Our results confirm previous results that although the KNP elephants are in a relatively good genetic condition, there is a loss of genetic variation between adults and juveniles. A high degree of genetic variation within a population is important for it to be genetically fit. In such populations certain individuals will be better equipped to cope with traits such as growth rate, survival and fecundity, and wildlife conservationists should establish the genetic structure of the population in order to ensure the long-term survival thereof. On the other hand, a population containing little genetic variation may be capable of resisting some threats to survive, but ultimately some factor will present itself to which most of the individuals have very little resistance owing to their similar genetic composition, increasing the probability of their extinction (Whyte 1993). The important characteristics of population size and reproductive efficiency are determined by historically established gene pools (Altukhov 1981).

The selection intensities were determined by values obtained for relative fitness, using the genotypic data in Table 2. The values of s are 0.691, 0, 0.895 for the AA, AB and BB alleles respectively and the initial B frequency is 0.02. Results from the simulation programmes indicate the disappearance of genetic variation of KNP elephants within 13 generations or approximately 200 years (based on age at puberty of 10–13 years and gestation period of 22 months). The results also indi-

cate that the B-allele could be lost within the next 12–13 generations. This result is concordant with that of Coetzee *et al.* (1993).

Contingency χ^2 analysis at all loci indicated no significant ($p<0.05$) differences in allelic distributions between the populations at four loci (ALB, EST-2, LDH-1 and LDH-2). This result (using BYOSYS-1) was confirmed with GENEPOP, and the relationships of the groups are depicted in Figure 2. The group from the 1993 culling programme is separated from the 1992 group (78% bootstrap value), with the juveniles of 1992 closer related to the 1993 elephants. This is probably coincidental because gene flow within and between the groups is expected to be high for family groups, and for populations that are not geographically isolated. It is conceivable that the dominant bulls in the area sired many of the offspring in both populations. This close relationship can be demonstrated by significant ($p<0.05$) differences at only four of 25 loci studied in the samples of 1992 and 1993, indicating that 84% of the allele distributions were similar. There are very similar allele distributions (i.e. allele frequencies; Table 2) for the respective groups, and the congruence is also reflected by the small genetic distance values of 0.004 (Nei, 1978) and 0.009 (Nei *et al.* 1998) between the populations.

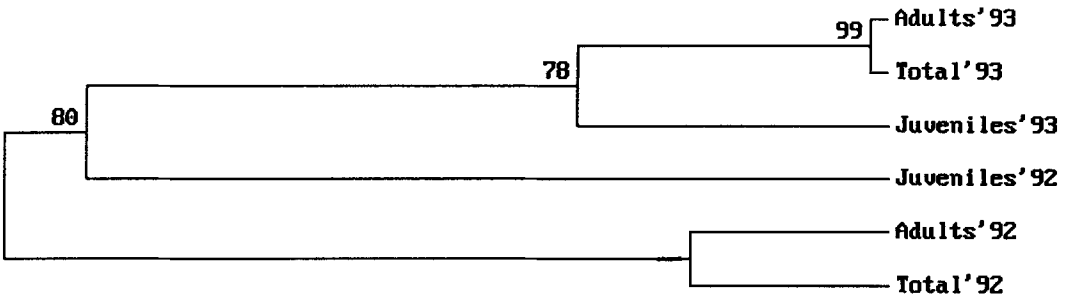

Figure 2 Phenogram produced by DISPAN (1000 bootstrap replications), using the genetic distance values of Nei *et al.* (1983). Numbers at nodes are bootstrap values.

The results of this study showed that the previous estimate of genetic variation in the KNP elephant population was slightly higher, but that a similar trend exists for predictions of the loss of genetic variation if the method of culling is continued. An increase in the KNP elephant population size has occurred since the culling programme was terminated in 1994. This increase could not be controlled by translocations owing to the high cost of such actions. We expect that culling or other alternative measures may resume, and will be employed to manage the overpopulated KNP elephants. We therefore recommend that the populations with rare alleles should be monitored over a period of time, and to exclude such bulls from translocation. Individuals with high genetic variation or rare alleles should remain in the KNP to contribute towards a higher genetic variation in the total population. Furthermore, the results for the elephants where large sample sizes were involved are important for the future monitoring of gene flow in populations to determine levels of inbreeding and crossbreeding, and to enhance the global information on wild animal diversity.

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