Mitochondrial DNA analysis of two southern African elephant populations

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Essop, M.F., A.J. Hall-Martin and E.H. Harley. 1996. Mitochondrial DNA analysis of two southern African elephant populations. *Koedoe* 39(1): 85-88. Pretoria. ISSN 0075-6458.

The modern view is that there are at most only two valid forms of the African elephant namely *Loxodonta africana africana*, the bush elephant, and *L.a. cyclotis*, the forest elephant (Ansell 1974; Meester *et al.* 1986). The Knysna elephant which was also described as a separate sub-species is now almost extinct. Plans to augment the remnant population by introducing other animals must take into account the taxonomic questions and issue of conserving elephant gene pools (Greig 1982a). Mitochondrial DNA (mtDNA) restriction fragment-size comparisons were performed on specimens from the Kruger National Park and the Addo Elephant National Park. If the Addo population's results are extrapolated to the Knysna population, it may be concluded that there is no genetic evidence for the Kruger and Knysna elephant populations to be considered as different sub-species.

Key words : *Loxodonta*, subspecies, mtDNA, Knysna, Kruger National Park, Addo Elephant National Park.

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Introduction

The two most southerly surviving herds of the African elephant are found in the evergreen high forest north of Knysna (approximately 400 km west of Port Elizabeth), and the Addo Elephant National Park a few hundred kilometres to the east (Smithers 1986). The Knysna elephant population occupies a dense forest area of approximately 3000 hectares, and has been under constant threat since the previous century (Thesen 1981). Hunting-licenses were readily granted during the 19th century, which led to elephant numbers declining to 30 - 50 in 1902 (Phillips 1925; Domisse 1951). Protective measures were introduced during 1908 with the Knysna elephants being declared Royal Game (Kinloch 1968). In spite of the strict protective measures, their numbers did not increase as expected. In 1970 they numbered eleven while in 1980 their numbers were given as only three (Greig 1982b). Numerous reasons, including poaching, and shooting by farmers concerned at damage caused to their crops, have been given for the decline of the Knysna elephants. However, it has also been argued that their decline may be due to a nutritionally deficient diet. Koen *et al.* (1988) has found that phosphorus/calcium ratio measured in Knysna elephants is significantly lower than that in the Kruger and Addo populations. The phosphorus/calcium ratio is relevant to the reproductive potential of animals, with lower ratios being associated with lower fertility. This could contribute to the reasons why the Knysna elephants, despite protective measures, did not increase in numbers to the same extent as did the Addo population.

Lydekker (1907) classified the Knysna elephant as a separate subspecies, *Elephas africanus toxotis*, using ear-shape as a distinguishing character. Claims were also made at the time that the Knysna elephants were the largest living elephants (Greig 1982a). In 1920, a licence was granted to Major P.J. Pretorius to kill a Knysna bull, to prove the theory that they were a larger, distinct sub-species (Kinloch 1968). Measurements in the field showed the bull to be much taller than other African elephants. Subsequent measurements done by the South African Museum in Cape Town showed however, that their height was not significantly different from other African elephants.

Using mtDNA fragment-size comparisons, we set out to determine whether the Knysna elephant population is genetically different from other African elephant populations. If it could be shown that they had significant genetic differences, it would provide some justification for conservation measures to ensure their survival as a distinct group of elephants.

Materials and methods

MtDNA analyses were performed on heart-tissue and muscle biopsies from nine elephants from the Kruger National Park and muscle biopsies from five elephants from the Addo Elephant National Park. Samples for the Knysna population were difficult to obtain, and therefore not included in this study. MtDNA was extracted, purified as previously described, and restricted with 12 restriction endonucleases (*Bam*HI, *Bcl*I, *Bgl*II, *Eco*RI, *Hind*III, *Nco*I, *Pst*I, *Pvu*II, *Sac*I, *Sac*II, *Stu*I *and Xba*I) for both the Kruger and Addo populations (Smith *et al.* 1971; Cummings *et al.* 1987). Restriction fragments were end-labelled using ^{32}P dCTP, separated by agarose gel electrophoresis and visualised by autoradiography (Ausubel *et al.* 1988). Restriction patterns were then compared in order to detect polymorphisms. Sequence divergence values were determined from the proportion of shared restriction fragments as previously described (Nei & Li 1979).

Results

All individuals of the Addo population gave identical restriction patterns for all 12 restriction endonucleases used. The Kruger population gave identical restriction patterns for nine restriction endonucleases, but three separate variants occurred for three restriction endonucleases, namely *Bam*HI, *Bcl*I and *Eco*RI (Table 1).

With respect to these Kruger polymorphisms, the Addo patterns corresponded to the more frequent Kruger pattern in two instances (*Bcl*I and *Eco*RI) and once to the less frequent pattern (*Bam*HI). Six of the Kruger elephants gave three restriction fragments (variant a) using *Bcl*I while the other three Kruger elephants gave only two fragments (variant b) with the same restriction endonuclease. All the Addo elephants gave three restriction fragments using *Bcl*I. The six elephants (variant b), have therefore gained an additional restriction endonuclease site, with the large restriction fragment of variant a being cleaved into two smaller restriction fragments. For *Eco*RI, four variants, and for *Bam*HI two variants were found. A sequence divergence estimate of 0.27 % was calculated for the Kruger population, and a value of 0.38 % for the combined elephant population (Kruger and Addo).

Table 1

Levels of mtDNA variation between Kruger National Park and Addo Elephant National Park populations

Restriction endonuclease	Number of cutting sites	Addo population	Kruger population
*Hind*III	3	a	a
*Nco*I	2	a	a
*Pst*I	7	a	a
*Bam*HI	4	b	a/b
*Bcl*I	3	b	a/b
*Bgl*II	3	a	a
*Eco*RI	7	b	a/b
*Pvu*II	4	a	a
*Sac*I	5	a	a
*Sac*II	2	a	a
*Stu*I	5	a	a
*Xba*I	3	a	a

Discussion

This restriction fragment data demonstrated a moderate degree of polymorphism (0.27 %) within the Kruger population, which is appropriate for an outbred population. The mtDNA sequence divergence estimate for the combined elephant population (0.38 %) is similar to that found within other southern African mammalian populations. O'Ryan *et al.* (1994) for example, recently found a 0.40 % sequence divergence estimate between sub-species of black rhinoceros, *Diceros bicornis*. The Kruger National Park elephant population numbered only 10 in 1905 but subsequently increased to approximately 8821 elephants in 1970 (Greig 1982b). The population has since then been maintained (through culling) at approximately 7 500 elephants which is considered to be within the carrying capacity of the Kruger National Park.

The complete monomorphism found in the Addo population is not surprising, since it is a small, isolated population, maintained at low numbers for a considerable time, which would allow for genetic drift to eliminate much of the genetic variability. The elephant population of the Eastern Cape was nearly exterminated earlier this century, and some elephants sought refuge in the thick impenetrable "Addo Bush" (Hall-Martin 1980). In 1926 there were only 16 elephants left, and a reserve was proclaimed for them. However, the elephants spent more time outside the reserve, which led to the Addo Elephant National Park being proclaimed in July 1931, with only 11 elephants remaining. The population has since then increased to approximately 225 individuals (Hall-Martin *pers. comm.*).

Although both the Kruger and Addo populations went through population bottlenecks earlier this century, the Kruger population's numbers were boosted due to a more rapid population expansion after the bottleneck together with immigration of elephants from neighbouring areas. This has resulted in a higher degree of genetic variation, as demonstrated by our mtDNA analysis.

The Addo population's results may be extrapolated to the Knysna population for historical and biogeographical reasons. Approximately 100 - 250 years ago, they formed part of a single continuous population ranging from the Western Cape in the south, to the Kruger National Park in the north (Fairall 1982). Elephants are very mobile animals, capable of covering distances greater than 200 kilometres in a matter of days (Hall-Martin 1987), hence genetic exchange would have taken place over the entire region. This is reflected in our data since all the restriction patterns found in the Addo population were present in the Kruger population. This suggests that the Addo population represents a genetic subset of the larger Kruger population. There is no support from the mitochondrial studies for the Kruger and the Knysna populations to be considered as different subspecies. They are more likely to be part of a continuous clade, with individuals from these localities being forced to seek refuge in impenetrable forests or bush after the arrival of the European settlers and hunters from 1790 onwards.

It may be noted that Lydekker's subspecific designation for the Knysna elephant was based on the examination of a single museum specimen and used only a single character, i.e. ear-shape (Lydekker 1907). The evolutionary equivalence observed among the southern African elephant populations we have examined, is in total agreement with the population structure at a much larger scale on the continent. Recently, Georgiadis *et al.* (1994) did a mtDNA analysis of ten savanna elephant populations from five African countries (including the Kruger population), which revealed evidence of a long distance gene flow over long periods. In view of the genetic continuity throughout *L. africana*'s geographical range, the costs to ensure the preservation of the Knysna elephants are not justifiable.

Acknowledgment

We thank the FRD for financial support and Dr C. Raath for collecting the study material.

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