African elephant genetics: request for samples

Alfred L. Roca,[1] *Nicholas Georgiadis* [2] *and Stephen J. O'Brien* [1]

[1] Laboratory of Genomic Diversity, National Cancer Institute, Frederick, Maryland, USA
[2] Director, Mpala Research Centre, Nanyuki, Kenya
email: roca@mail.ncifcrf.gov *and* njg@mpala.org

The Laboratory of Genomic Diversity and the Mpala Research Centre have been conducting a continent-wide genetic survey of African elephants. We found evidence supporting species-level genetic distinctions between forest and savannah elephant populations in Africa (Roca et al. 2001). We would like to expand this survey by using additional genetic markers and by sampling populations from additional regions of Africa as well as additional individuals from sites previously undersampled. A broader sampling of elephant populations, would provide much additional information on the evolution, natural history, biogeography and taxonomy of African elephants—particularly hybridization, which will be useful for conservation efforts on their behalf. We request the assistance of AfESG members, researchers, conservationists and others who may be able to collect elephant samples from anywhere in Africa.

Summary of our published findings

In a recent publication (Roca et al. 2001), we examined DNA sequence variation in four nuclear gene introns (a total of 1732 base pair) in African elephants from 21 populations across Africa, using DNA extracted primarily from dart-biopsy tissue samples (Karesh et al. 1989). The genetic distance between African forest elephant and savannah elephant populations was large, corresponding to 58% of the difference in the same genes between elephant genera *Loxodonta* (African) and *Elephas* (Asian). There were multiple fixed nucleotide site differences between forest and savannah African elephants. Genetic evidence for hybridization between the two was limited to Garamba (Democratic Republic of Congo), which was the only intermediate forest–savannah habitat zone sampled. Analysis of individual gene haplotypes (alleles) indicated that, outside the hybrid zone, gene flow across the forest–savannah boundary was not detected. Along with previously established morphological and habitat distinctions, the genetic evidence supported the recognition and conservation management of two distinct African species: *Loxodonta africana* and *Loxodonta cyclotis*.

Sampling locations

We hope to expand and build upon this work by adding more populations of elephants and by using additional genetic markers. We have adequate sampling from the following locations: Dzanga–Sangha Forest Reserve, most of Botswana, Kruger National Park, south-western Zimbabwe, northern Namibia, northernmost Tanzania, Amboseli and most of central Kenya.

We welcome additional samples from the other locations in our study as well as from any locations that we have not previously sampled. Our top priorities are for samples from Zambia, Congo (especially south of the Congo River), and all nations in Africa west of Cameroon. We are also looking for samples from Malawi, Mozambique (especially northern), and central and southern Tanzania; from Ethiopia, Sudan and Chad; from any additional forest location; and from intermediate habitat regions or putative hybrid zones. In summary, we are looking for samples from all but the locales listed in the previous paragraph.

Types of samples preferred

In general, we have extracted DNA of excellent quality from all of the following: skin samples collected by biopsy darts of the type designed by Karesh et al. (1989); blood or tissue from planned culls or immobilizations; and samples of tissue, even dried tissue, from elephant carcasses resulting from natural death or from hunting.

However, if it is not feasible to collect tissue of any kind in your area, we welcome dung samples, from which we have also been able to extract DNA. Note that from almost any tissue source the quantity and

quality of DNA extracted is much better than from dung. But dung samples are nevertheless useful where tissue is not available.

All tissue and dung samples should be collected as fresh as possible. When feasible, the samples should derive from an unambiguously identified individual. Any available information should be recorded regarding the individual elephant from which the sample is taken, such as name or identification number, sex, age, herd, location, date collected, storage medium. However, if individual identification is not possible, then record the location, date collected, storage medium and any other known information.

In locations with large elephant populations, and where feasible, collect one individual sample per herd, to give us an overall view of the population. However, if the population of elephants in a location is very small, then collect more than one individual sample per herd, but make sure that this information is recorded along with other available information.

Samples from individuals of uncertain geographic origin, such as those sold in bushmeat markets, are not as useful for biogeographical studies.

Samples of tissue (muscle, organs, skin)

Samples of muscle, organs, skin or other soft tissue should be placed in ethanol. Having successfully extracted DNA from samples stored in alcohol, we prefer the use of 90–100% ethanol. While soft tissue can also be preserved in buffers, such as the TES buffer that is used to store blood (detailed below), we have had better success in extracting DNA from samples in ethanol, and it is our preferred storage medium. However, the shipment of ethanol is highly regulated; therefore, allowing the ethanol to evaporate before shipping or storing in other media may be appropriate in some cases.

Tissue that is metabolically active, such as from muscle or organs, is best, although almost any tissue, including skin, will be adequate. Avoid surface tissue directly exposed to sunlight, air or soil. Even a small amount of tissue sample, 1 cm^3 or even smaller, can provide sufficient DNA, although several cubic centimetres is preferred. The volume of 90–100% ethanol should be at least four times greater than that of the sample, and the tube should be filled to the top or close to it with the ethanol.

Regardless of storage medium, it is helpful to cut slits in the tissue to allow for better penetration of the fluid. It is also important to minimize cross-contamination of samples by using different blades or thoroughly cleaning blades between samples.

Any sturdy leakproof screw-cap tubes can be used to store the samples, which if possible should be kept cold or preferably frozen until shipped.

Samples of blood

Blood can be collected from live animals in blood collection tubes or other tubes containing anticoagulants such as EDTA (preferred) or ACD. If mixed only with anticoagulant, the blood must be kept refrigerated and shipped as soon as possible. It can also be mixed with an equal volume of TES buffer ('Easy Blood'), in which case it can be stored for longer periods and even kept at room temperature; TES buffer is 100 mM Tris, 100 mM EDTA, 2% SDS (sodium dodecyl sulphate).

This buffer, when mixed in equal part with fresh blood in anticoagulant, will lyse the red and white blood cells but will protect the DNA and inhibit nuclease activity and microbial growth. This solution is used in field situations where no centrifugation or refrigeration is available. Once the samples are back in the lab, refrigeration or freezing is recommended for long-term storage.

Appropriate quantities of TES can be dispensed into vials for transport to the study site. Use a large enough vial to allow space for an equal volume of blood. Alternatively, the dry components can be weighed into plastic vials for transport and later mixed with the appropriate amount of sterile distilled water at the study site. However, this requires weighing out microgram amounts of each chemical.

TES buffer

From stock solution (in ml) for . . .	100 ml	500 ml
Sterile distilled water	50	250
0.5 M Tris HCl pH 7.5	20	100
0.5 M Na_2 EDTA (pH must be adjusted to 8 to dissolve EDTA)	20	100
20% SDS (sodium dodecyl sulphate)	10	50

From dry chemicals, g per 100 cc (sterile distilled water to final volume of 100 ml, pH adjusted to 8)	
Tris base (MW = 121.2)	1.2 g
EDTA Na_2 (MW = 372.2)	3.7 g
2% SDS	2.0 g

Samples of dung

A sterile tongue depressor (a new one for each different sample, to avoid cross-contamination) or any similar sterile item can be used to collect a dung sample at least several grams or cubic centimetres in size. Ideally a fragment concentrating on the surface of the dung can be collected, so that it contains a higher proportion of sloughed-off intestinal cells, thereby yielding more DNA. If this is not feasible, or if the outside has had long exposure to sunlight, an internal portion can be used.

Different ways of storing the sample are possible. One possibility is for the dung, especially if fresh or wet, to be placed into a sturdy, leakproof screw-cap tube, with the tube then filled to the top with 90–100% ethanol (ethanol volume at least 4 times the sample volume), capped and briefly shaken to allow the ethanol to penetrate. However, shipping samples in ethanol may be problematic (see above). An alternative is to place the dry dung in a screw-cap tube with silica-gel beads at the bottom, separated from the dung by filter paper (there is no problem if some silica-gel beads are in contact with the dung). We can supply the tubes with silica-gel beads.

Other details

Appropriate permits should always be obtained before collecting. If possible, please contact the Laboratory of Genomic Diversity before collecting the samples, especially if ethanol will be used as the storage medium. We can provide you with the necessary materials for sample collection and with information on required documentation and permits, and we will pay for shipping costs.

A broad sampling of elephant populations will allow the tools of molecular genetics to uncover the evolution, natural history, biogeography and taxonomy of African elephants, providing much information useful for their conservation. We thank those who have provided samples previously, and those who are willing to assist in the future.

Contact information

Alfred L. Roca, PhD
Stephen J. O'Brien, PhD
Laboratory of Genomic Diversity
National Cancer Institute – FCRDC
Building 560, Room 11-10A
Frederick, MD 21702-1201, USA
tel: +1 301 846 1299
fax: +1 301 846 6327 or 1909
email: roca@mail.ncifcrf.gov

Nicholas Georgiadis, PhD
Director, Mpala Research Centre
PO Box 555, Nanyuki, Kenya
tel: +254 176 32752
fax: +254 176 32750
email: njg@mpala.org

References

Karesh, W., Smith, F., Frazier-Taylor, H. 1989. A remote method for obtaining skin biopsy samples. *Conservation Biology* 1:261–262.

Roca, A.L., Georgiadis, N., Pecon-Slattery, J., O'Brien, S.J. 2001. Genetic evidence for two species of elephant in Africa. *Science* 293:1473–1477.