Darwin Initiative for the Survival of Species

Half Year Report Form

Project Title	Population genetic study of forest elephants (*Loxodonta africana cyclotis*) in central Africa.
Country	Gabon
Organisation	University of Cardiff, Cardiff, Wales, UK
Project Ref. No.	
Report date	*June 2004*

1. Outline progress over the last 6 months against the agreed baseline timetable for the project (if your project has started less than 6 months ago, please report on the period since start up).

We have collected over 400 samples from Gabon, Republic of Congo and Central Africa Republic (CAR). We have visited 10 sites in Gabon, 2 in Republic of Congo and 1 in CAR. Twelve blood and tissue samples were collected during elephant captures to attach radio-collars. DNA has been extracted in duplicate from all faecal samples at CIRMF.

A 630bp fragment of mitochondrial DNA (mtDNA) was amplified using primers MDL3 and MDL5 (Fernando *et al*., 2000). The amplified fragment comprised 109bp of cytochrome b, 135bp of Thr and pro tRNAs and 386bp of the Control Region (CR). The Polymerase Chain Reaction (PCR) conditions that were optimised for the laboratory in UGENET, CIRMF had to be re-optimised in Cardiff. The original number of cycles was increased from 30 to 40, the extension time was also increased from 45 seconds to 1 minute and DNA volume (from faecal extracts, concentration unknown but less than 1ng) was doubled from 1 to 2µl. 28 individuals from various sites in Gabon and two from the Republic of Congo, all presumed to be *Loxodonta africana cyclotis* or forest elephants, were sequenced and aligned with previous elephant sequences published by Eggert et al., (2001), Nyakaana & Arctander, (1999), Debruyne et al., (2003). Preliminary phylogenetic analysis currently in progress was restricted to 386bp of CR. We suspected that we may have amplified some nuclear copies of the CR (Numts), so to address this potential problem we cloned PCR products from 5 individuals (5 to 10 clones per individual) and sequenced 17 samples of a fragment of 494bp cytochrome b (L15024/H15516). All analysis is in progress.

2. Give details of any notable problems or unexpected developments that the project has encountered over the last 6 months. Explain what impact these could have on the project and whether the changes will effect the budget and timetable of project activities. Have any of these issues been discussed with the Department and if so, have changes been made to the original agreement?

We planned to have most of the samples by the end of 2003, but this has not been the case because the sampling network was difficult to organise: including making contact with each collaborator at different sites, sending and subsequently receiving tubes in time to start processing. However sampling is going on and most of the contacts have been successful. We expect material from at least 13 sites in Gabon and 5 other sites in Republic of Congo, CAR and Equatorial-Guinea.

The only change we have made to account for this delay is to divide in two my time in Cardiff: instead of

8 months consecutively, I stayed 4 months to have time to extract 141 samples from 3 important sites: Conkouati-Douli reserve, Nouabale-Ndoki National Park in Republic of Congo and Dzanga National Park in CAR. The main issue we have with samples is that sloughed cells on the outer layer of elephant dung degrade more quickly on silica gel than when collected into the more expensive RNAlater. Silica gel was the field storage system first sent out to sites. We have noticed it can preserve cells efficiently, but nuclear DNA for more or less 2 months, after this time we have less chance to extract enough DNA for the following genetic analysis. Consequently, I had to process all samples rapidly otherwise I would have "lost" the samples. This is a distinctive feature of elephant dung stored on silica gel because it's not the same problem with mandrill (*Mandrillus sphinx*), gorilla (*Gorilla gorilla gorilla*) or chimpanzee (*Pan troglodytes*) dung on silica gel.

Excepting the fact that I started the mtDNA analysis before the microsatellite study (due to the issue mentioned above and the fact that this study is more intensive than mtDNA analysis), project activities still remain exactly the same.

3. Are there any other issues you wish to raise relating to the project or to Darwin's management, monitoring, or financial procedures?

No.

Please send your **completed form by 31 October each year per email** to Stefanie Halfmann, Darwin Initiative M&E Project Manager, Email: stefanie.halfmann@ed.ac.uk