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DARWIN INITIATIVE

DARWIN SCHOLARSHIP REPORT (PROGRAMME 2003-2004)

Project Title: **Population genetic study of forest elephants**
(Loxodonta africana cyclotis).

Country: Gabon

Organisation: University of Cardiff, Cardiff, Wales, UK

Report date: *April 2005*

Background

I was identified as a potential Darwin Scholar after working as a research technician in laboratory techniques on the Darwin Initiative project (Ref. No. 162/08/044) entitled “Genetic differences between populations of western lowland gorillas throughout their range and specifically within Gabon”. During the three years of the Darwin Initiative collaboration with Cardiff University, I received training in population genetic analyses of DNA variability. I learned techniques suitable for DNA extraction from degraded gorilla (*Gorilla gorilla gorilla*) faecal and shed hair samples from the field; microsatellite and mitochondrial DNA genotyping, cloning and sequencing using automated sequencers and data analysis methods for phylogenetics and population genetics.

As a Darwin scholar, I aimed to consolidate the Darwin Initiative grant. The additional period of the Darwin Scholarship served to carry out an in depth training in the theory, as well as the practice of conservation genetics, in reinforcing the links between field and laboratory and my integration within the conservation community as a recognised expert. With all these acquired skills, I also aimed to develop my own research project on forest elephant population genetics. The same problems, which confront gorillas, also affect forest elephant populations (poaching, habitat destruction and a lack of socio-ecological information on this critical taxon) in Central Africa, particularly in Gabon. The elephant project was planned to continue after the scholarship in collaboration between my home institution CIRMF (Gabon) and my host institution, Cardiff University.

Scientific Project

Introduction

Gabon’s extensive rainforests still harbour important populations of large mammals, and elephants contribute significantly to the maintenance of the forest ecosystem, modifying structure and dispersing seeds. The loss of significant numbers of elephants is likely to be detrimental to the ecosystem’s integrity. Although large tracts of Gabonese forests are included in logging concessions, sustainable forestry exploitation practices are being instigated and a major effort is underway following the creation of a network of 13 national parks, encompassing 11% of the country’s area. This presidential initiative shows Gabon’s commitment to maintaining and protecting its biodiversity, and highlights a pressing need for qualified Gabonese conservation scientists. Effective conservation ultimately depends on trained individuals, and it is within this framework that I am studying the conservation genetics of the forest elephant in my country, as well as in other contiguous central African countries.

Aim

To provide essential information through genetic analysis on population and social structure, dispersal and phylogeography of the central African forest elephant (*Loxodonta africana cyclotis*) to underpin urgently required management and action plans, and to define its taxonomic status.

Objectives

This scholarship year served to instigate a larger research programme, designed to:

1. Investigate the phylogeography of forest elephant across its habitat range in central Africa.
2. Examine genetic variability and define levels of gene flow between central African elephant populations.
3. Provide my training in conservation biology, population genetics and molecular ecology at Cardiff University, UK and at the Centre International de Recherches Médicales de Franceville (CIRMF), Gabon.

Sampling strategy and locations

Elephant faeces collection began in 2000, and is still on going. Approximately 500 samples are already available for analysis. This fieldwork is carried out in collaboration with WCS (Wildlife Conservation Society) and the National Parks programme, and through long-standing collaborations in neighbouring countries (Cameroon, Congo, Equatorial Guinea, Central African Republic and Democratic Republic of Congo). 21 sites (Figure1) across central Africa have been targeted, to look at the phylogeography and the population genetic structure, and identify potential barriers (historical and geographical) to gene flow. In addition, four sites (Lopé and Ivindo National Parks in Gabon, and Nouabalé-Ndoki and Conkouati-Douli National Parks in Republic of Congo) have been targeted for more extensive sampling. These four locations show different habitat types and more fine-grained genetic analyses will help to examine the role of seasonal resources in elephant behaviour, the differential use of forest types, the spatial utilisation of habitat areas, and the social structure of the forest elephant.

Material and methods

A 630bp fragment of mitochondrial DNA (mtDNA) was amplified using Asian elephant primers MDL3 and MDL5 (Fernando *et al.*, 2000) optimised for african forest elephants at CIRMF. 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 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. Individuals from various sites in Gabon, from CAR and the Republic of Congo, all presumed to be *Loxodonta africana cyclotis* or forest elephants, were sequenced and aligned with previous elephant (savannah and forest) sequences published by Eggert *et al.*, (2001), Nyakaana & Arctander, (1999), Debruyne *et al.*, (2003). Phylogenetic analysis was restricted to 386bp of CR. I suspected some nuclear copies of the CR

(Numts) may have been amplified, so to address this potential problem I 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).

Results

This study included 104 *mtDNA* CR sequences that I combined with savannah and forest sequences from Genbank. Total sequence number is 205. Phylogenetic analysis shows no significant separation between savannah and forest elephants. There are at least 4 weakly supported groups:

1. Forest elephants from central Africa with some forest and savannah elephants from West Africa.
2. Savannah elephants from eastern and southern Africa with a small number of savannah elephants from West Africa.
3. Forest elephants from central Africa mixed with forest and savannah elephants from West Africa.
4. Savannah elephants from southern and eastern Africa mixed with forest elephants from DRC.

This result is novel, as it indicates repeated contact between forest and savannah clades, which has not been seen using other genetic markers.

483 bp of cytochrome *b* was aligned and analysed for 64 elephant and 3 mammoth sequences. Of the elephant sequences 47 were African forest elephants, 8 were African savannah elephants and 9 were Asian elephant sequences. Twenty-eight of the African forest elephant sequences were produced in this study. The remainder of the sequences were obtained from GenBank. African forest, African savannah, Asian and mammoth separate out into four distinct lineages with bootstrap values of 100, in accordance with other genetic studies (Roca *et al.*, 2001; Comstock *et al.*, 2002). No admixture between African forest and savannah elephants was evident using this data set, in contrast to the above results for the control region.

Perspectives

Phylogeographic studies of elephant populations across several national boundaries will reveal the existing levels of gene flow between populations throughout Central Africa. My close collaboration with WCS Gabon, and other conservation organizations will allow the data I generate to be directly implicated in elephant conservation and management plans. The data generated during my study will also be used as a baseline from which to monitor future trends in elephant population genetics, as forests become more and more fragmented.

Gabon has a pressing need for qualified conservation scientists. Effective conservation depends on trained dedicated individuals. This project will provide me (a national scientist) with a thorough training, ultimately to doctoral level in conservation biology, population genetics and molecular ecology. To my knowledge, I will be only the second Gabonese national expert in this area. The majority of the laboratory extraction and screening procedures is carried out in Gabon at CIRMF's Unit of Genetics of Tropical Ecosystems (UGENET), which is the only molecular ecology laboratory of this

kind within the central equatorial rain forest belt, with additional laboratory work in Cardiff, UK. The project thus has an important capacity building and technology transfer element, which will be of long-term benefit not only to Gabon but also to the central African region.

My experiences

Contact network

. The study of forest elephant genetics is done on two scales: local (Gabon) and regional (Congo basin). I organised an extensive sampling campaign. The collaboration with WCS provided important contacts in Gabon, Central African Republic, Republic of Congo and Democratic Republic of Congo, all coordinated by Bryan Curran who is in direct contact with me. For other sites such as Conkouati-Douli National Park in Republic of Congo, Dr Benoit Goossens put me in touch with the NGO HELP Congo and the conservator of the Park.

The first important action of my project was to establish a national and international contact networks. All contacts are done by electronic mail, phone or direct contact. During the previous gorilla project, I had already met some people who made developing my contact network easier.

Training

My training in molecular techniques during the previous project gave me the capacity to transfer techniques from Cardiff University laboratory to UGENET at CIRMF. At the same time, I trained people at CIRMF as well as in Cardiff.

****in the lab***

I taught students basic knowledge regarding good laboratory procedure in a molecular biology laboratory: fundamental notions of security, the wearing of coat and gloves, handling dangerous and toxic reagents, and potentially infectious biological material, and finally the use of delicate apparatus. In addition, I trained students, lab technicians and teachers from USTM, national university in molecular techniques such as dung extraction, PCR amplification and electrophoresis on agarose and acrylamide gels.

At Cardiff University, I had the opportunity, during my scholarship, to train a post-doctoral researcher who intended to work on black rhinoceros. He came to the lab specifically to learn how to extract DNA from dung samples. Dr B. Goossens introduced me to him and I showed him our extraction technique, and we successfully amplified the DNA extract. I also worked with the team studying the genetic of Guanaco and Vicuña in the framework of a Darwin Initiative programme in Peru, training them in SSCP electrophoresis techniques. I also helped Fairus Jalil on his thesis project on the phylogeography of 3 primate species in Sabah (Malaysia).

****in the field***

Field training was capital because we use non-invasive sampling techniques. It was crucial, since I did not do all the sampling myself, to train people to do it properly. At Lopé National Park and Conkouati-Douli National Park, I taught field teams directly how to collect dung samples. For those teams not able to benefit from first hand training, I prepared a manual (Appendix I) detailing the correct way of identifying and collecting fresh dung samples. This avoids wasting time and money because sampling done incorrectly (e.g. non-respect of sample/preservative ratio) leads to fungus growth.

Fieldwork

At these same two sites (Lopé National Park in central of Gabon and Conkouati National Park on the west coast in Republic of Congo), fieldworkers and I collected at least twelve fresh dung samples. We went with fieldworkers through the forest looking for fresh (24h) elephant dung from what we take a small piece on the top layer and put it in a tube containing a dry (silica gel) or a liquid (RNAlater) preservative.

This fieldwork allowed me to better appreciate all the problems linked to the samples, confronting teams collecting dung; it also allowed these teams to learn about the use of the samples collected and meet the laboratory researcher personally.

It is necessary to know and understand the habitat of your study species and the best way to do that is to be there.

Presentations

In February 2004, I presented a poster at Cardiff University (Appendix II). In June 2004 during a field-training programme, I gave a talk at the training centre in Lopé National Park for national field technicians.

Popular presentations

Two papers are in preparation for the general public. One will be published in the annual magazine of Tusk Trust, a British funding organisation. The other one will be published in a Gabonese conservation paper, "Cri du Pangolin".

One scientific paper is in preparation.

Applications for funding

I acquired considerable experience of writing applications for funding, with my supervisors' assistance; all of which were successful.

- Tusk Trust: British funding NGO
- FORINFO: French cooperation
- BES: British Ecological Society
- USFWS: American funding organisation (US Fish and Wildlife Service)

My future work

As indicated above, the Darwin scholarship leads directly into my thesis on forest elephant genetics. I am still waiting for samples from Cameroon and DRC, and in June I plan to sample in Equatorial Guinea. WCS Gabon teams will complete sampling as remaining sites in Gabon.

I will continue teaching students about molecular techniques in UGENET lab at CIRMF, and fieldworkers on how to collect dung samples, at Lopé's training centre.

I will give seminars at the national university (USTM), talking about the link between molecular studies and conservation genetics.

I will present my work to national and international NGOs and potential sponsors, in Gabon or abroad, and also at international scientific meetings

I will collaborate with national institutions (CNPN, CENAREST) and NGOs (WCS, WWF) using my data to establish management strategies of elephants within Gabon's National Parks. CNPN is a national committee overseen all 13 National

Parks in Gabon. CENAREST is the national research centre coordinating all scientific programmes.

I will continue to expand the international network I have developed. I am already in contact with the Coopération française via FORINFO one of a financial supported of my thesis, with U. S. Fish and Wildlife Service, another important financial support. New collaborations will arise with eminent researchers as S. Nyakaana (Uganda, East Africa), H. Siegismund (Copenhagen, Denmark) and researchers implicated in the new Darwin Initiative project on Asian elephants in Malaysia (headed by Pr M. Bruford and Dr B. Goossens, Cardiff University).

In addition, at the end of 2006 or mid 2007, I will co-organise an international workshop on the conservation of forest elephant in Franceville and for which I would solicit your support.

Suggestions and perspectives for developing Darwin activities in Central Africa.

I suggest that the Darwin Initiative maintains long-term contact with individuals such as myself, who have benefited from support, as it is a real problem for students or researchers from southern countries to access money and to achieve their work. The Darwin Initiative with the Scholarship gives the opportunity to provide training in development, management and evaluation of scientific projects with the aim to consolidate a country's capacity in conservation genetics and molecular ecology. The main problem is to be affiliated to a British institution, what is not always possible, especially for French speaking countries in Africa. Hence, my question: would it be feasible to create a kind of trust branch of the Darwin Initiative in UK to an institution or NGO in Gabon? A previous Darwin scholar or scientist could manage it. Students or young researchers would then have more opportunities to develop much-needed projects in conservation, since equatorial, tropical ecosystems, as substantial biodiversity reservoirs, need management strategies. Collaboration with expert teams, such as WCS, WWF are a considerable support in the field. WCS at Lopé (Gabon) have a well-organised training centre where they train young people as field technicians for censusing plants and animals, or for the management of National Parks. Indeed, since the creation of the 13 National Parks in 2002 by the President of Gabon, there is a strong demand for a national work force to achieve this presidential goal. UGENET and the station at Lopé (CIRMF), under the Darwin Initiative 3 years project on western lowland gorillas, trained some students from USTM who are presently employed at WCS working for the National Parks. Unfortunately we are limited by financial support. A second Darwin project in Gabon continues this objective but comes to the end this year.