THE DARWIN INITIATIVE IN THE GABON: MOLECULAR ECOLOGY AND CONSERVATION OF WESTERN LOWLAND GORILLAS

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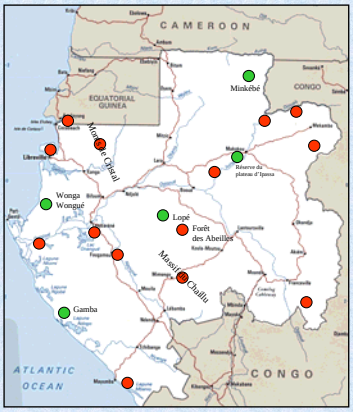
Western lowland gorilla, Gabon

Background

The overall aim of the Darwin initiative is to promote global biodiversity conservation through research, training and education. The research focus of this program in Gabon is to use population genetic data to create a national long-term conservation strategy for the western lowland gorilla. Gabon is an important centre of biological diversity and harbours the largest remaining western lowland gorilla populations in the wild. Information from patterns of genetic variation at different spatial scales will help define the current status of gorilla populations and provide genetic input into a long range management plan for the subspecies.

Over the past 16 years, intensive research at the CIRMF primate research station (Station d'Etudes des Gorilles et Chimpanzes) in the Lopé reserve has provided a solid body of ecological knowledge. Shed hairs have been collected from over 6 identified family groups during the past 10 years. This source of DNA provides us with a fascinating opportunity to explore social organization and group dynamics at the local level. At larger geographical scales, genetic analysis of gorilla hair and faecal material across the Lopé reserve and from a network of candidate protected areas throughout Gabon will allow us to examine intraspecific variability and genetic structure at the regional and landscape level.



Map illustrating the network of WCS collection sites throughout Gabon. Existing protected areas are indicated by green circles. Sampling sites representing candidate areas for protection are indicated by a red circle. This work is being coordinated by WCS and is a part of a ongoing inventory program (2000-2001) to assess candidate areas for protection throughout the country.

View of the Lopé reserve from the field station (SEGC)

Experimental approaches

Genetic studies of free-ranging threatened and endangered species often rely on a non-invasive source of genetic material. In the present study, we have developed successful protocols for the amplification of mitochondrial and nuclear DNA markers from shed hairs recovered from nest sites and fresh faeces.

A fresh gorilla nest

Faecal collection at the Lopé

Molecular markers

Mitochondrial DNA: Amplification and sequence analysis of ~ 330 bp of the left-hand hypervariable domain of the mitochondrial control region from single hairs and faeces.

Human microsatellite loci: Co-amplification of six polymorphic loci in two triplex combinations (D1S550, D5S1457 and DXS6810; D8S1179, D21S11 and HUMFIBRA).

Sex identification: Molecular sexing using the amelogenin system.

Hair

We are following a forensic approach to classifying gorilla hair root morphologies. There are three distinct growth cycles in mammalian hair:

anagen (active growth phase)
catagen (breakdown phase)
telogen (resting phase)

Hairs can also be categorised by the presence or absence of germinal tissue attached to the root .

This directly affects nuclear DNA yield and m-sat PCR success

Faeces

Control region amplification was compared using four preservation methods:

(a) DETs (DMSO/EDTA/Tris/Salt solution)
(b) Absolute ethanol
(c) Silica beads (Type II 1/8"Sigma)
(d) RNA-later solution (Ambion).

Two different extraction methods were also compared:

(a) The Qiagen stool extraction kit
(b) Diatomeceous earth-based extraction method

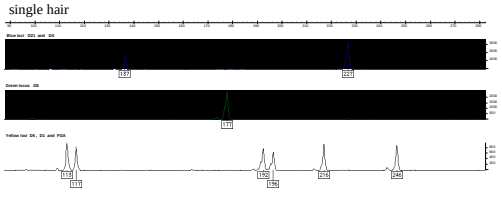
Telogen: no germinal tissue	**Telogen:** minimal germinal tissue	**Telogen:** ample germinal tissue	**Anagen:** sheath attached
			
44% nest hairs 1.6% plucked hairs	37% nest hairs 38% plucked hairs		0.4% nest hairs 57% plucked hairs
Nest Hair 0% m-sat PCR success 5 hairs per extract (n=12)	**Nest Hair** 1.7% m-sat PCR success 1 hair per extract (n=36) 53.6% m-sat PCR success 8 hairs per extract (n=28)	**Plucked Hair** 30.2% m-sat PCR success 1 hair per extract (n=130)	**Plucked Hair** 97.9% m-sat PCR success 1 hair per extract (n=30)

Repeated PCR amplification of faecal samples from captive gorillas stored for (a) 1 week; (b) 5 weeks room temperature (N=4), and for (c) fresh field-collected samples (N=2).

	Qiagen extraction method				Diatomeceous earth			
	DETs	Ethanol	Silica	RNA-later	DETs	Ethanol	Silica	RNA-later
1 week	+++	++	+++	+++	+++	++	++	+++
5 weeks	+++	+++	+++	+++	++	++	++	+++
Field	++	nt	+	+++	+	nt	-	+

Amplification success: + (0 – 50%); ++ (50 – 75%); +++ (75 – 100%)

Genetic profile of a Lopé silverback co-amplified at six loci from a single hair



Technology transfer and conservation education

In the past year, the Darwin initiative has been responsible for:

(1) within country technical training
(2) transfer of appropriate molecular technologies
(3) development of a conservation biology course at the Université des Sciences et Techniques de Masuku (USTM) in Franceville.

In the final trimester of the 1999-2000 session, a 6 week course in basic conservation biology was opened up to all second year biology undergraduate students at USTM. The course covered basic topics in conservation biology including: definition and measurement of biodiversity; an introduction to population biology; rates and causes of extinction; the problems of small populations; island biogeography and principals of reserve design.

Un nouveau cours ouvert à tous à l'USTM sur:
LA DIVERSITÉ BIOLOGIQUE
ET SA CONSERVATION

Un projet en collaboration entre le Centre International de Recherches Médicales de Franceville, L'Université de Cardiff, et l'USTM, Franceville.

Two outstanding students from the course were offered a short internship at CIRMF. Both completed an intensive five week intership introducing them to both laboratory and field aspects of molecular ecology. Although training of other students and CIRMF personnel will continue throughout the second and third years of this project, the Gabonese research specialist on the project (Mireille Johnson-Bawe) will ultimately assume responsibility for all technical aspects in molecular ecology at CIRMF.

Implications for the present study

Understanding population genetic structure and patterns of gene flow at different spatial scales is essential to the formulation of an effective conservation management strategy.

At the local scale: a fine-scale genetic analysis of patterns of relatedness within social groups will provide novel insights into group size, ranging behaviour, dispersal and social organisation of western lowland gorillas.

At the regional scale: a cross-section of genetic variability across the Lopé reserve will give us a regional profile of population structure and gene flow across a range of habitat types.

At the national scale: Genetic data from populations throughout Gabon will allow us to examine genetic variability and genetic structure of natural populations across a network of candidate protected areas. A geographic analysis should also allow us to identify potential management units for conservation within the context of a recently completed pan-African study of gorilla genetic variation (Clifford *et al*., in prep.).

Lowland forest in the Lopé reserve