

Darwin Initiative for the Survival of Species

Annual Report

1. Darwin Project Information

Project title	<i>Conservation biology and Genetics of the Western Lowland Gorilla in Gabon</i>
Country(ies)	<i>Wales, UK; Gabon</i>
Contractor	<i>University of Wales, Cardiff</i>
Project Reference No.	<i>08/044</i>
Grant Value	<i>148,133</i>
Start/Finishing dates	<i>October 1, 1999 - Sept 30, 2002</i>
Reporting period	<i>Fiscal Year 2 (April 1, 2000 - March 31, 2001)</i>

2. Project Background

Briefly describe the location and circumstances of the project and the problem that the project aims to tackle.

1). **Location of project.** Gabon is an important centre of tropical diversity with substantial tracts of closed canopy forest still remaining. These forests are some of the most botanically diverse in Africa with a rich assortment of endemic plants. Gabon is also a stronghold for some of the largest viable populations of large mammals remaining - census estimates from the 1980s indicate that Gabon's forest were home to as many as 35,000 gorillas and 64,000 chimpanzees and around 60,000 elephants. Forest refuges present in the three montane areas of the country during the Pleistocene era may be one of the factors responsible for high regional diversity.

2). **The problem.** With the decline in the oil industry, Gabon's forests and their associated fauna are increasingly under threat to logging pressure. With a population of a little over 1.2 million, Gabon has few resources with which to plan conservation measures and monitor their impact. Furthermore, there is a considerable lack of national expertise in the domains of applied ecology, wildlife management and conservation. Furthermore, research and training opportunities for students and scientists are limited. At present, higher education in the biological sciences is limited to the national science university *Université des Sciences et Techniques de Masuku* (USTM) and the national training school for natural resource managers *École Nationale des Eaux et Forêts* (ENEF).

Before the beginning of this grant, neither institute had a formal program in conservation biology and both institutes remain limited in the qualifications they offer. There is presently no bachelor's program in Biology at USTM and there are no facilities for teaching students practical skills in molecular biology or field ecology. After completing the first two years of their training, students have to go abroad to finish their degrees. Education at the ENEF (which is the national training school for

the Gabonese Fish and Wildlife Department) provides technical training for Fish and Wildlife managers and is considered to be at a lower standard than at the university.

3). **Program rationale.** *The Centre International de Recherche Médicale à Franceville (CIRMF)* and associated field station *Station d'Etudes des Gorilles et Chimpanzés (SEGC)* are the only research centres within the country that have the capacity to sustain an applied ecological and genetic research program. Accordingly research centres have played an integral role in the development of the Darwin Initiative in Gabon. Although CIRMF is principally a medical research facility, it has one unit in molecular ecology *Unité de la Génétique des Ecosystèmes Tropicaux (UGENET)* through which the Darwin program operates. SEGC is the centre for field operations at CIRMF and for the Wildlife Conservation Society's (WCS) program in Gabon. WCS are also heavily implicated in the Darwin Initiative and are largely responsible for the coordination and collection of gorilla hair and faecal samples within Gabon and elsewhere in Africa. The University of Wales in Cardiff (UWC) is a nationally recognised centre of excellence in both teaching and research. In particular, Dr. Bruford's laboratory has strengths in conservation biology and genetic resources management and was thus selected as a suitable UK institution for training national scientists and for facilitating the transfer of appropriate technologies to Gabon. Through collaborative links set up through the Darwin Initiative, UWC was also well placed for (a) creating and ultimately transferring a program in conservation biology to USTM (b) establishing a research program in gorilla conservation genetics.

3. Project Objectives

State the purpose and objectives (or purpose and outputs) of the project. Please include the Logical Framework for this project (as an appendix) if this formed part of the original proposal or has been developed since, and report against this.

- a) **Research:** Development of a national research program on gorilla genetic diversity. The study aims to provide genetic data on gorilla social structure, dispersal behaviour and biogeographical history of the gorilla species complex as a whole. In the long term the Darwin Initiative in Gabon has sought to lay down the foundations for a durable molecular ecology research program applicable to large tropical mammals. Work has initially focused on the western lowland gorilla but could be expanded to other species in the future.
- b) **Technology transfer and training:** Training of one full time Gabonese technician in molecular ecology techniques. Facilitate her participation in scientific meetings, the USTM teaching program and the supervision of student projects. Additional trainees including another technical staff at CIRMF (Simon Ossari) and several student interns recruited from the conservation biology course at USTM. Next year, we hope to hold a lab practical for third year students in basic molecular techniques.
- c) **Conservation education:** Establish a university teaching module in conservation biology and integrate this course into the national university curriculum. Provide research opportunities at CIRMF and SEGC for students during the long summer vacation. Organise and teach field courses in collaboration with WCS/SEGC on basic ecological/field survey techniques.

Have the objectives or proposed operational plan been modified over the last year and have these changes been approved by the Darwin Secretariat?

No the objectives have remained the same.

4. Progress

Please provide a brief history of the project to the beginning of this reporting period. (1 para.)

The UK training period for the Gabonese technician (Mireille Johnson-Bawe) was completed successfully at UWC. Many important techniques were acquired and subsequently transferred to CIRMF. These included:

- (i) identification and optimisation of suitable molecular markers
- (ii) extraction and amplification of DNA from shed hair and faecal samples
- (iii) analysis of genetic polymorphisms in the mitochondrial genome through SSCP and sequence analysis
- (iv) Development of an automated system for detecting allelic variation in microsatellite loci.
- (v) visualisation and interpretation of these repeat polymorphisms using silver staining and automated fluorescent based technologies.

In the first year of this grant, a 6 week course in conservation biology was successfully launched at the national science university and two student interns passed 4 weeks training at CIRMF/SEGC during the summer of 2000.

Summarise progress over the last year against the agreed baseline timetable for the period. Explain differences including any slippage or additional outputs and activities.

Although the objectives of the project have remained the same throughout the past year, much emphasis in the past year has been placed on objectives 2 and 3 because of the shortage of incoming field samples. Samples from the Lope reserve including 6+ identified family groups within the study zone adjacent to SEGC are presently being genotyped. However samples from 20 or so candidate protected areas in Gabon are slow in arriving. This has been due to delays in field operations, staff shortages and an apparent absence of gorilla sign which may be due to reduced population sizes. With the recent acquisition of additional field samples across the entire range of the gorilla we anticipate making rapid research progress although it should be said that this Pan-African sample set cannot entirely replace the shortfall of samples at the national scale.

Microsatellite PCR amplification and typing protocols have been successfully established in laboratories in Gabon and Cardiff. Analysis of mitochondrial sequence variation has begun on gorilla samples collected from wild populations in Gabon and throughout Africa. Training of the Gabonese technician has continued throughout the second year of this project and key skills in and out of the laboratory have been

established. A student intern is scheduled to spend part of this summer in the CIRMF laboratory learning molecular skills.

An extended nine-week course in conservation biology was taught to approximately forty 2nd year students at USTM. This course consisted of a combination of lecture style sessions, directed practical exercises and open seminars where speakers from Gabon and abroad were invited to share their real world experiences. An optional one-day field course taught principally by WCS/SEGC scientists was provided for all participating students. A more intensive one-week long field course was also taught during the Easter holidays to 10 USTM students. Students were required to pass a 2-hour exam in order to gain a diploma. One student intern has been taken on for a 4-week training period at UGENET this summer. A curriculum for a 3rd year program has also been developed in anticipation of the eventual integration of the conservation biology course into the *licence* (bachelors) degree program that is anticipated will begin this autumn (2001).

Provide an account of the project's research, training, and/or technical work during the last year. This should include discussion on selection criteria for participants, research and training methodologies as well as results. Please **summarise** techniques and results and, if necessary, provide more detailed information in appendices (this may include cross-references to attached publications).

Genetic analysis. During the course of the last year, gorilla microsatellite typing protocols have been established and optimised in Gabon and Cardiff University. These protocols involve the automated analysis of variation at 7 microsatellite loci and a sex-determining marker in gorilla hair and faecal samples. In Cardiff, microsatellite typing is carried out on the ABI 377 using a four-dye system and at CIRMF on the Amersham ALF Express single dye system. Since microsatellite analyses are being run concurrently on two different systems we have developed allelic ladders from identified individuals with known allele sizes to control for potential variation between the two system.

Optimisation of DNA extraction and amplification methodologies has also been achieved for both gorilla and hair faecal samples. Owing to the difficulties of working with DNA from shed hair, a forensic approach developed by UWC student Kathryn Jeffery has been used to screen hair samples and identify those that are most likely to maximise PCR amplification success. Co-amplification of multiple microsatellite loci within the same PCR reaction has helped conserve precious DNA and increase sample throughput. We are now in the process of genotyping hair samples from identified gorilla family groups. These samples are very valuable and have been collected over the past 10 years by several research scientists at the SEGC site in the Lope reserve. It is hoped that data from this project will provide novel insights into gorilla ranging behaviour and genetic structure at different spatial scales.

At larger geographic scales we have begun to receive samples from WCS survey teams collecting gorilla hair and faeces from throughout Gabon. Further to these samples, we have recently been able to capitalise on existing samples from a Pan-African sample set that has already formed the basis for a preliminary study of gorilla mitochondrial DNA variation (Clifford et al., submitted). Sequence analysis of nucleotide variation in the mitochondrial DNA hypervariable control region at UWC is ongoing. Further work on this sample set, which will initially rely heavily on mitochondrial DNA variation, will provide us with information on the past population history and geographic structure of genetic variation within western lowland gorillas

across their entire range. Information from this kind of approach can also help identify genetically unique management units for conservation.

Teaching and training. The conservation biology course was strengthened this year at the university and expanded into a 9-week course. Students who attended a minimum of 7 weeks of classes were awarded a certificate of participation. A diploma was awarded to those who passed the exam. Practically all students who participated in the course took an optional one day field course in methods for estimating animal abundance using a software package (LOPES) recently developed by WCS (Walsh, 1998). This one-day sampler was taught within the grounds of CIRMF by WCS/SEGC staff. In contrast, the one-week field course was held at the Lopé reserve in Central Gabon, some 400 km from Franceville. Because of financial and logistical limitations (housing, transportation, supervision), this one-week field course had to be restricted to ten students. Students were selected through an essay assignment and required to write reports at the end of the field course. Both field courses were carried out in collaboration with WCS/SEGC scientists and USTM faculty were encouraged to participate.

The one week field course which was held at SEGC in the Lopé reserve was designed to introduce students to a range of methods for studying mammals (elephants, buffalo, primates, large carnivores, small mammals). Techniques that they were introduced to included radio-telemetry, track and faecal analysis, direct observation, camera trapping and small mammal live trapping. Students were assigned a group project for the week and asked to critically evaluate the results at the end of the field course as well as write a report of what they had learnt. This field course was enormously successful and despite the high cost (£1000) should be an integral part of the education program. Outside the one-day field course held at CIRMF, the Lopé field course was the first time that most students had access to practical field training.

Plans for next year's course are already in place. A nine-week conservation biology course will be formally integrated into the USTM higher education program and be taught initially to 3rd year (*licence*) students. A one-week field course for all participating licence students will also be held jointly with other faculty on campus. An introductory seminar and field day is also planned for 2nd year students. Two faculty members at USTM have been identified as instructors of the conservation biology course in future years and will work closely with the Darwin research associate next year to ensure the effective transfer of the conservation biology course to the department. This next year will also be rather unusual in that it will be the first time that the university has held classes at the level of the licence. In the event that the university is not able to launch a third year program, 2nd year students will be taught as before. Either way, the most important task for next year is to work with USTM lecturers to ensure that this course will be taught in future years.

Training of the Gabonese research specialist Mireille Johnson-Bawe has basically followed the evolution of the automated microsatellite system at CIRMF. Mireille has taken charge of this facility and has become recognised as expert by her peers. She has also gained experience in optimising PCR conditions for amplifying microsatellite markers and in DNA extraction techniques for non-invasively collected material. Mireille has also been trained in laboratory management skills and in the supervision and training of other laboratory staff at CIRMF. She has also developed her communication skills and given several presentations at lab meetings in Cardiff, at a scientific meeting in London (November, 2000) and to the conservation biology class

at USTM. She has actively participated in all aspects of the university teaching program and now acts as local liaison between teaching staff, administrative offices (dean and vice-rector) and CIRMF.

In terms of training student interns at CIRMF, interns have been exposed to a range of molecular techniques widely used to tackle problems in applied biology. Emphasis during the training course was placed on ways in which molecular biology can be used to better understand ecology, population processes and biogeographical history or organisms.

a) Molecular component

- DNA extraction methods
- PCR principles and the amplification process
- molecular markers in the mitochondrial and nuclear genome
- sequence analysis and use of restriction enzymes
- design of a molecular diagnostic for discriminating species; haplotypes etc.

b) Ecology component

- using a compass, GPS system and a map; radiotelemetry
- censusing methods (botanical surveys and assessing animal abundance)
- Collection and organisation of data
- Integrating data into a GIS system

A weekend workshop was also held for executive members of a local NGO based in Okandja, central Gabon. The main purpose of this workshop was to explore ways of critically evaluating program objectives. NGO group members were broken up into discussion groups that focused on each of the stated project goals of the organisation. Group members were asked to discuss strategies for evaluating the feasibility of their respective projects (school and adult environmental education, rearing antelope or other local animals for meat consumption; native plant nursery). It is hoped that this workshop will aid NGO members in writing grant applications and attracting external funding.

Discuss any significant difficulties encountered during the year.

Teaching was halted on several occasions this year due to university staff strikes. At one point during the course, teaching was suspended for 7 weeks making course continuity a challenge. These strikes also had deleterious effects on the whole academic year with students being put under pressure to complete their studies and teaching/exams being extended into the long summer vacation. The effect was so severe that we have had significant problems finding a 4-week window with which to offer a summer training course. This problem has not been fully resolved and may continue in the future.

Obtaining fresh samples of dung and hair from gorilla populations around the country has also been more of a challenge than was originally anticipated. Samples sizes are still rather limited which will compromise what can be done with the data set in Gabon. However, this loss is more than offset by access to a large Pan-African data

set so that the research emphasis of this project is now more likely to shift towards a continent-wide rather than detailed regional analysis. In terms of interpreting phylogeographic patterns this shift can only serve to strengthen the study, particularly as so little is known of the population genetic structure of wild gorilla populations. We are currently sequencing a larger region of the hypervariable domain than was examined in the original study and focussing on sample populations that have not yet been examined. It may also be possible to use microsatellites at larger geographic scales if sample sizes permit (Kahuzi-Biega, Central African Republic, South Cameroon, Lopé Reserve) to look at patterns of gene flow and regional differentiation at the nuclear level. However, lack of access to any real samples over the past 6 months in Gabon has set back the research progress of this project. Lack of communication within Gabon continues to be a problem and the relative isolation of CIRMF presents a lot of logistical difficulties, particularly with the ordering and delivery of chemical and biotech supplies.

Has the design of the project been enhanced over the last year, e.g. refining methods, indicators for measuring achievements, exit strategies?

Yes we have tried to maintain a flexible approach in that our research objectives may need to be adapted to the samples that are available. Optimisation of microsatellite typing protocols have taken a great deal of time and represent a significant technical challenge because of the poor quality of nuclear DNA in hair and faecal remains. Because of the challenges of optimising two molecular typing facilities in parallel, we decided to split the research work between the two institutions so as to avoid unnecessary repetition of optimisation procedures. The division of labour was as follows:

- CIRMF: DNA extraction from faeces; amplification of mitochondrial DNA and microsatellite loci from faeces; DNA purification.
- UWC: DNA extraction from hairs; amplification of mitochondrial DNA and microsatellite loci from hair samples; mitochondrial DNA sequence analysis.

Teaching material and methods have also been refined as a result of student evaluations and feedback from faculty and vice-chancellor who attended the 2001 course. Assigned written reports and exam responses have also helped to refine teaching methods.

Present a timetable (work plan) for the next reporting period.

April - October 2001, Gabon: Collection, extraction and analysis of samples collected from Gabon.. CIRMF technician completes optimisation of amplification of microsatellite loci from faeces. Extraction and archival of incoming samples from WCS. Transfer of hair samples to UWC.

July - December 2001, UK: microsatellite analysis of selected family groups from the Lopé reserve. This work is being done in collaboration with UWC graduate student Kathryn Jeffery. Amplification and sequence analysis of mitochondrial DNA from

hair and faecal samples from Gabon and across a range of gorilla populations throughout equatorial Africa (Nigeria, Cameroon, Equatorial Guinea, Congo, Democratic Republic of Congo (DRC), Central African Republic, Uganda). Gabonese technician to transfer to Cardiff to gain further experience in sequence analysis (September-November, 2001).

January - March 2002: teaching of third year course in conservation biology. Training of two USTM faculty and transfer of course materials to the department.

Partnerships

Describe collaboration between UK and host country partner(s) over the last year. Are there difficulties or unforeseen problems or advantages of these relationships?

CIRMF is the only molecular research institute in the country where molecular techniques are routinely used and so is the only location in Gabon where this work could be done. The Unité des Ecosystèmes Tropicaux is gaining strength both in terms of personnel and resources making it a productive work environment. Isolation of CIRMF in Franceville does however present problems as there is little opportunity for scientific exchange outside the immediate research community and receiving reagents is sometimes very difficult.

SEGC is part of CIRMF and integral to our work in Gabon. This field station has provided opportunities for Darwin personnel to liaise directly with field scientists, has co-ordinated the field sample collection and has acted as a tremendous resource base with which to co-teach a field course. WCS is committed to providing samples for the Darwin program. WCS has also provided the Darwin initiative with considerable logistical support during the field course in terms of staff time, accommodation and local transportation for students.

UWC/USTM – A memorandum of understanding was put in place this year between UWC and USTM. A formal visit from the Dean of USTM in May of this year also facilitated exchange of ideas on staff development for the conservation course and exchange visits of USTM faculty in the near future.

WCS – The Darwin project interacts with the WCS national conservation program in terms of sample collection, teaching and field training. WCS has also published an excellent technical manual on conservation research in African rain forests which has aided field training.

Has the project been able to collaborate with similar projects in the host country or establish new links with / between local or international organisations involved in biodiversity conservation?

A collaboration between UWC and the Institut de Recherche Tropicale (IRET) has been offered to offer technical and logistical help to a UWC student interested in finding natural predators in agricultural crop systems in Gabon.

A list of opportunities for voluntary participation with local NGOs involved in conservation has been distributed to USTM students present at the exam.

The Gabonese environmental newspaper *Cri du Pangolin* have expressed an interest in offering students internships in environmental journalism next year.

The UWC research associate visited the IRET field station located in Gabon's only biosphere reserve this year in order to assess its potential for student field courses in the future. The site is currently under renovation by funds from the European Union and hopefully maybe a suitable basis for student projects in the future.

Two Peace Corps volunteers approached the Darwin project in Gabon wishing to exchange ideas and collaborate on building an environmental education centre between Kalamatou and Lastourville. Once established this site may provide internship opportunities for students interested in environmental education.

5. Impact and Sustainability

Discuss the profile of the project within the country and what efforts have been made during the year to promote the work. What evidence is there for increasing interest and capacity for biodiversity resulting from the project? Are satisfactory exit strategies for the project in place?

Integration of course into national university curriculum; support from Dean and Vice Chancellor has been very important

Willingness of direction at CIRMF to support the Darwin Initiative and student internships

Transfer of technical capacity and molecular ecology expertise to the Gabonese research specialist on the project. At the end of the Darwin Initiative, Mireille will become a permanent member of staff at CIRMF and will provide technical support to incoming researchers. As she has gained considerable expertise in the principals of molecular ecology and conservation genetics she will also be encouraged to develop research interests of her own.

Training of university professors and the eventual transfer of the conservation biology course to the biology department will ensure the ultimate integration of conservation biology into the national university's core curriculum.

CIRMF and SEGC will continue to support university education in terms of laboratory support, field courses, invited speakers etc.)

There may be further training opportunities and academic exchanges for Gabonese students but these will involve seeking additional external financial support.

6. Outputs, Outcomes and Dissemination

Please expand and complete Table 1. **Quantify** project outputs over the last year using the coding and format from the Darwin Initiative Standard Output Measures (see website for details) and give a brief description. Please list and report on appropriate Code Nos. only. The level of detail required is specified in the Guidance notes on Output Definitions which accompanies the List of Standard Output Measures.

Table 1. Project Outputs (According to Standard Output Measures)

Code No.	Quantity	Description
4A	30-50 students	Conservation biology course taught to university

		undergraduates
4B		9 week course
5		Training of full time Gabonese technician on the project
6A	10 students	One week field course in animal survey techniques
7	1	Weekly course handouts, field course handout, course poster, slides, power-point presentations; CDs with entire course contents including invited speakers distributed to biology faculty in Franceville and Libreville and WCS. Posters in English and French on the Darwin Initiative are on display at UWC, CIRMF and USTM.
8	2	UWC postdoc: 22 weeks; UWC faculty: 3 weeks
11 A/B	3	Mitochondrial phylogeography of gorillas and book chapter (Stephen Clifford); forensic approach to evaluating PCR amplification success of shed hair (Kathryn Jeffery).
21	1	Conservation biology course established at the university

Explain differences in actual outputs against those agreed in the initial 'Project Implementation Timetable' and the 'Project Outputs Schedule', i.e. what outputs were not achieved or only partly achieved? Were additional outputs achieved?

Outputs generally on track with those outlined in the initial grant application. Obviously some outputs will not be achieved until the end of the third year. More efforts will be made to communicate the Darwin project to the local and national press.

In Table 2, provide full details of all publications and material produced over the last year that can be publicly accessed, e.g. title, name of publisher, contact details, cost. Details will be recorded on the Darwin Monitoring Website Publications database which is currently being compiled. Mark (*) all publications and other material that you have included with this report

Table 2: Publications

Type * (e.g. journals, manual, CDs)	Detail (title, author, year)	Publishers (name, city)	Available from (e.g. contact address, website)	Cost £
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Provide details of dissemination activities in the host country during the year. Will these activities be continued by the host country when the project finishes, and how will this be funded and implemented?

Information on the project and personnel is on the UWC website.

Posters on the project are on display at UWC, CIRMF and USTM

CDs of course have been provided to key USTM faculty in Libreville and Franceville free of charge

An oral and poster presentation of the Darwin project was made at the European Federation of Primatology meeting in London in November, 2000.

Durability of dissemination activities:

Teaching: The university course it is hoped will form a permanent module in the third year curriculum.

Production of a text book to accompany the course in French would be an ideal product with which to complete the Darwin Initiative in Gabon.

Mireille will take up a full-time position at CIRMF when she finishes the program and will be encouraged to develop her own research interests as well as continue to be the technical lynch-pin of the laboratory at UGENET.

7. Project Expenditure

Please expand and complete Table 3.

Table 3: Project expenditure during the reporting period

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- Highlight any recently agreed changes to the budget and explain any variation in expenditure where this is +/- 10% of the budget

8. Monitoring, Evaluation and Lessons

Discuss methods employed to monitor and evaluate the project this year. How can you demonstrate that the outputs and outcomes of the project actually contribute to the project purpose? i.e. what indicators of achievements (both qualitative and quantitative) and how are you measuring these?

Student evaluation sheets were handed out at the end of each course to obtain feedback on their perception of the course and its value in their curriculum. Course content and structure has been modified during the last two years in response to student critiques. Exams and assigned papers have also been used to assess and modify course material.

CIRMF's independent scientific review council of Gabonese and international experts have independently reviewed the teaching and research activities of the Darwin Initiative at CIRMF and congratulated all CIRMF personnel on their motivation and success.

- Are there lessons that you learned from this year's work and can you build this learning into future plans?

Communication and feedback: evaluations, particularly from students, have been instrumental in designing and modifying the course to suit their needs.

Curriculum development: cutback on material and focus on principal points. The more case studies the better as it is important that students can see how conservation theory/methodologies are applied in practice.

Practical work: More time is needed for students to assimilate practical exercises. Practical field work is crucial to student development. A practical molecular biology component for third year students would also be a considerable asset to students on the 3rd year course. USTM has currently no means of offering molecular and field ecology courses and any molecular training would have to be based at CIRMF for the time being.

Networking with other organisations in the country has been very rewarding. We need to do more work in this area outside Franceville as much as possible.

Ensuring continuity in the course of course is crucial to any development project. Selecting candidate instructors for the course has not been very straightforward.

We have experimented with different teaching media with mixed results. Slides were a disaster due to problems with lighting conditions and a problematic projector. Power point works well but is dependent on the availability of a projector from WCS.

Lecture style: Successful lecturing still remains a question of finding out what students need, already know and are used to. Changes to traditional methods need to be done in moderation. More time for directed study and practical work is needed and less on lectures. Encouraging students to ask questions and think critically is the most important challenge.

9. Author(s) / Date