

Darwin Initiative for the Survival of Species

Half Year Report Form

Project Title	Genetic diversity and management implications for high Andean guanaco populations in Peru.
Country	Peru
Organisation	Cardiff University
Project Ref. No.	162/12/022
Report date	21st October 2005

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).

This report covers the period between 01/04/05 and 30/09/05 and began with Darwin Trainee Jorge Rodriguez (JR) returning to Lima on 31/03/05. The Cardiff PDRA Ciara Dodd (CD) travelled to Lima on 01/06/05 for 6 weeks to deliver the second conservation biology course and troubleshoot in the laboratory. During this time the objectives for subsequent six month of the project were outlined as: a) running the Conservation Biology Course; b) identifying the remaining laboratory work and training goals; c) setting out the details for the PHVA.

a) Conservation Biology Course

The second one-month course in Conservation Biology was held at Facultad de Medicina Veterinaria, Universidad Nacional Mayor de San Marcos, Lima. The structure of this course was based on the previous year's course, covering the same key topics. An intensive period of teaching of core lectures took place in the first 2 weeks and was delivered in English, with transcripts in Spanish, by PDRA (CD) and Coordinator Mike Bruford (MWB). As with the previous course, the core lectures covered aspects of conservation biology, population biology, threats to biodiversity, conservation genetics, and population and habitat viability assessment (PHVA). The second two weeks of the course consisted of invited guest lecturers from governmental, university and conservation organisations to discuss many aspects of conservation projects and legislation within Peru. Two special topic days were organised to which members of the public were invited. These days were advertised in the local newspapers, at the university and on the CONOPA website.

A one day workshop was organised to the International Potato Centre (CIP) in Lima. Staff from the centre delivered a series of talks on many aspects of potato (and its relatives) conservation, diversity and genetics. This workshop provided the students with an excellent opportunity to visit an international research centre and to learn about the importance of the conservation of plant genetic resources.

Applicants for the course were invited from governmental organisations such as INRENA, CONAM, CONACS, Peruvian NGOs and international conservation organisations. Nineteen students were enrolled onto the course after their applications were evaluated by CONOPA (Jane Wheeler (JCW) and Domingo Hoces (DH)). The number of students from each organisation was: INRENA 3, CONAM 2, Museo de Historia Natural de Lambayeque 1, CONOPA 2, UNALM 1, Facultad de Medicina Veterinaria San Marcos 2, Universidad Alas Peruanas 3, Asociación Craccidae 1, CONACS 2 and Conservation International 1.

At the conclusion of the course all of the lectures were compiled on to a DVD together with additional background information that was given to all students and course speakers.

Sixteen course participants were awarded a diploma and three a certificate of attendance at the end of the course. To obtain a diploma, students were required to provide satisfactory input to the course (minimum of 70% attendance) and successfully complete and deliver three coursework exercises: i) individual presentation about an endangered species; ii) group presentation about a current and

proposed management of a protected area; iii) individual presentation of a scientific paper. The students PHVA modelling exercise was assessed by MWB at the end of week two of the course. Students that did not satisfactorily complete the assessments were awarded a certificate of attendance. Coursework exercised were assessed by CD and JCW.

b) Laboratory work and training

The guanaco populations in Yanaque, (Moquegua) and Vilani, (Tacna) were sampled in December 2004 and the samples were brought to Cardiff in July 2005 for analysis. The second Darwin trainee Katherine Yaya (KY) arrived in Cardiff on 14th July for eight months of laboratory training. This is longer than initially anticipated, partly due to the availability of affordable airfares and to enable KY to receive adequate training in data analysis after the completion of the laboratory work. Since her arrival in July, KY has been trained in faecal DNA extraction, PCR, sequencing and sequence analysis. She has also received basic training in automated microsatellite multiplex PCR and gel analysis which will continue in greater detail in the next 6 months. Currently she is focusing on optimising the extractions for Calipuy using modified protocols such as CTAB. She began MWBs undergraduate Conservation Biology module at the beginning of October.

The priority areas for lab work during this period have been: to continue the sequencing of 10 individuals per guanaco population, where available, including the two new populations of Moquegua and Vilani; continue faecal microsatellite amplification of 20-25 individuals per guanaco population, where available; continue to optimise faecal DNA extraction from samples which have previously failed, particularly those from Calipuy; continue to optimise SSCP separation of guanaco mitochondrial DNA haplotypes on silver-stained gels.

During this period, the ability to successfully amplify microsatellite multiplexes from DNA extracted from blood and faeces, visualised on silver stained gels has been achieved and further work to optimise this will not be necessary, since the skill to achieve this is now present in CONOPA. Significant progress has been made with SSCP analysis of mtDNA haplotypes, although this still needs further optimisation which is being carried out in Lima by JR. This analysis has been problematic as outlined in section 2.

An undergraduate veterinary student from Cornell University, Sara Gomez-Ibañez (SI) arrived at CONOPA in June for a 2 month placement to gain experience in many aspects of camelid medicine and research. SI chose CONOPA as her training placement as a direct result of the Darwin Initiative project, information about which she obtained from the project website (<http://www.cardiff.ac.uk/biosi/research/biodiversity/staff/dodd.html>). As part of her placement she was trained in the laboratory by JR in PCR, SSCP and gel electrophoresis, using techniques that he learned from the Darwin project training.

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?

DNA extraction from faecal material has been successful in the majority of cases using the Qiagen Stool Kit. However individual samples and/or populations such as those from Calipuy have proven problematic and need to be extracted several times or using revised procedures. Despite some earlier success with extractions from the Calipuy population, more individuals are required particularly for microsatellite analysis. The texture/plant content of the faeces from the Calipuy guanacos appears to be different to the other populations that have been sampled, and it is possible that inhibitory plant compounds are being co-extracted with the guanaco DNA that are preventing the samples from amplifying. One solution could be to use the CTAB buffer method which was designed for use with plant DNA to remove potential inhibitors from the plant tissues. Drawing on the experiences of colleagues in the laboratory who have successfully used this method with particularly difficult samples, KY is currently attempting extractions with CTAB to try to resolve this problem. However, if this population continues to fail, it may be necessary to resample the animals.

Although progress is being made towards separating sequence haplotypes using SSCP analysis, the availability/cost of specialised equipment in Peru for this type of analysis is proving problematic. Improvements in band separation are being achieved for example by running gels in the fridge as a replacement for a cooled gel rig. Further optimisation is required to achieve single base pair separation of haplotypes and this work is continuing into the next six month period. However, the advent of cheap sequencing in Korea through Macrogen may result in the need for SSCP analysis of haplotypes becoming redundant for future projects in CONOPA.

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

Monitoring and evaluation

Weekly laboratory meetings have taken place in Cardiff with MWB. These meetings provide the opportunity to update progress of the project as well as to discuss any problems with MWB and other lab members, drawing on their expertise to help resolve problems. Lab talks and a journal club run regularly after lab meetings and a weekly departmental seminar takes place, attendance at which is required by students/trainees in the laboratory. These activities enable group discussion of current scientific papers, provide an informal environment for practicing the delivery of presentations, broaden the knowledge base of lab members and keep them informed about the research activities of group members. These activities are particularly valuable for the Darwin Trainee during their UK visit.

Since her arrival in July, KY has been trained and supervised in the laboratory by CD. CD and KY meet several times per week to discuss progress, to plan work and to develop strategies for resolving problems.

Regular email contact is maintained between project members so that progress and potential problems can be discussed and resolved quickly. This is particularly valuable when group members travel abroad.

A project meeting was held between CD, MWB, JCW and the CONOPA team on 18th June 2005 to discuss progress to date, work which is outstanding and to set out the structure and planning for the PHVA. The PHVA three day workshop is provisionally scheduled for 5th - 7th April 2006, using the PHVA held in Malaysia for the orang-utan as a model (DI project 09/016).

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