Darwin Initiative for the Survival of Species: Molecular tools for promoting biodiversity in rainforest fragments of Borneo

2nd Annual report

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Front cover shows *Mycalesis kina*, an species endemic to Borneo, adversely affected by habitat disturbance that opens up the canopy.

Darwin Initiative for the Survival of Species

Annual Report

1. Darwin Project Information

Project title	Molecular tools for promoting biodiversity in rainforest fragments of Borneo
Country(ies)	Sabah
Contractor	University of Leeds, UK
Project Reference No.	162/10/025
Grant Value	£106,814
Start/Finishing dates	1st April 2001 for three years
Reporting period	1.4.01 – 31.3.02

2. Project Background

Tropical conservationists face three major challenges in identifying priorities for conservation in the face of limited funding resources and the continuing loss of biodiversity: (1) tropical communities contain many cryptic species that are highly distinct genetically yet cannot be distinguished using traditional morphological methods. This is especially true of invertebrates, which constitute over 90% of biodiversity. Thus in many cases, even in well-studied taxa such as butterflies, inventories of invertebrate biodiversity are based on 'morphospecies', which have no taxonomic validity and thus are very poor estimators of biodiversity; (2) limited resources need to be targeted towards those species with the highest conservation value, but most tropical countries lack the molecular genetic skills required to identify those species which have the greatest phylogenetic uniqueness and so contribute most to biodiversity; (3) isolation of populations within forest fragments can limit the dispersal of individuals, so reducing genetic diversity within populations and increasing the likelihood of local extinctions. Effective conservation in fragmented landscapes requires estimates of gene flow patterns within and among habitat fragments, in order to minimize the isolation of populations and consequent loss of biodiversity. Yet the molecular genetic tools for estimating gene flow patterns are almost completely lacking in tropical countries. This is a matter of urgent concern because in the near future, most remaining tropical forest will occur as fragments scattered among agriculture and urban development. Conservationists in temperate regions are well aware of the importance of size and isolation of habitat fragments for species survival, but this has rarely been considered in tropical communities. This is unfortunate given that a high proportion of tropical species are dependent on forest. The State of Sabah (Borneo) is the poorest financially in Malaysia and the vast majority of its income is generated through conversion of rainforest into oil palm plantation and other forms of silviculture. This increasingly leaves patches of rainforest interspersed among oil palm and other plantations. These rainforest patches may contribute significantly to the conservation of rainforest biodiversity and some have been gazetted as Virgin Jungle Reserves to protect them from further disturbance. However resources for protection are highly limited and the choice of

patches to preserve is largely arbitrary, because forest managers lack the means of establishing priorities for patches based on key criteria (species diversity, representation of species of high conservation value and importance within a network of local populations). This lack of vital information also excludes forest managers from making informed recommendations as to the size and placement of forest patches to be preserved in future agricultural developments.

3. Project Objectives

The purpose of the project is, in consultation with Sabahan forestry researchers and managers, to allow the development of strategies that balance conservation of rainforest biodiversity with agricultural development to meet local community requirements. The project aims to attain the following Measurable Outputs:

1. Training of two Sabahan graduate biologists (Darwin Research Fellows) at Leeds and York Universities in (i) molecular techniques for identifying species and quantifying genetic diversity and (ii) ecological techniques for sampling and monitoring biodiversity in forest patches. This will include training in butterfly taxonomy and identification, cladistics and phylogenetic analysis, laboratory techniques for molecular genetic analysis, censusing techniques, database management and writing of reports and scientific publications (Code 4B and 5; March 2004).
2. Production of a comprehensive quantitative inventory of butterfly species in different sized forest fragments (Output Code 10) and a manual of genetic techniques (Output Code 10). Production of a fully labelled and annotated collection of specimens (Code 13B) and a computer-based database (Code 12A) to be housed at Universiti Malaysia Sabah and a field guide (Code 10) to assist with species identification, classification and recording (Output 10) (March 2004).
3. Production of two educational packages (pamphlets, 35mm slides, posters and leaflets), with narration in English and Malaysian, one to assist in teaching of molecular taxonomic techniques to local scientists, the other to assist in interpreting the consequences of forest fragmentation to local communities (Code 7; October 2000).
4. UK staff to spend a combined total of 28 weeks per person in Sabah assisting with data collection, training the Darwin personnel in the field and liasing with staff at collaborating institutions (Code 8; April 2001 - March 2004).
5. Establishment of 400 study plots in 20 forest fragments for long-term monitoring of butterfly biodiversity (Code 22; October 2001).
6. Evaluation of the relationship between forest fragmentation (size and isolation) and the diversity and conservation value of forest-dwelling butterfly species. Additionally, to estimate gene flow and genetic diversity within and among populations of selected butterfly species (10 papers to be submitted to peer-reviewed journals by March 2004; Code 11B).
7. Presentation of results of the project at meetings of the British Ecological Society, the Royal Entomological Society, UK and the Sabah Society, and organisation of research seminars and workshops at Universiti Malaysia Sabah to disseminate results and stimulate the development of new projects using molecular genetics techniques by the local scientific community (Code 14B; 10 presentations and two workshops by March 2004).
8. Presentation of results using the Sabahan and British local and national media where appropriate, also dissemination via local student networks and international networks including the World Wide Web (Code 15; at least 1 press and radio

release in host country and overseas by March 2004 and Code 17; Web Page established by December 2001). Production of management recommendations in consultation with the Sabah Departments of Forestry and Wildlife and the Forest Research Centre Sabah (Code 9; March 2004). Enabling of two collaborators from Sabah (Dr Maryati Mohamed and Dr Chey vun Khen) to visit Durham for two weeks each, to gather information on teaching and use of molecular genetic techniques (Code 6; March 2003 and March 2004).

There has been no alteration to these objectives or to the operational plan during the reporting period.

4. Progress

This project grew out of our previous Darwin Initiative project (Biodiversity of butterflies in tropical rainforests of Sabah, Borneo) as a direct result of observations made during our visits to Sabah and discussions with staff at the Institute of Tropical Biology, Universiti Malaysia Sabah and at the Forest Research Centre, Sabah. The first year of the project went well: two Sabahan graduate biologists were recruited and completed training in ecological techniques. Fieldwork sites were established in Sabah. Darwin Fellow 1 commenced training in molecular genetics at the University of York and Darwin Fellow 2 commenced collection of data and biological material in Sabah. In addition, we trained a further Sabahan biologist (Mr Nasir Abd Majid) in ecological sampling techniques including forest inventory, butterfly capture and identification skills. The two Darwin Fellows also received additional training in forest inventory techniques, which they put into practice by collecting data from each of the forest fragments to allow changes in vegetation structure and composition to be included in the analytical comparison between fragments.

During the current reporting period, all but two key milestones were reached: Darwin Fellow 1 completed training in molecular genetics at York, including construction of a DNA-based phylogeny for all sampled species of the genus *Mycalesis* (one of the most species-rich genera of butterflies in Borneo, with a high proportion of endemic species). Darwin Fellow 1 then commenced collection of data and biological material in Sabah, whilst Darwin Fellow 2 completed collection of data and biological material in Sabah and commenced training in molecular genetics at York: this included training in the theoretical basis of molecular genetic analysis, followed by first-hand experience of DNA extraction from butterfly material, amplification using PCR techniques, sequencing of PCR-products and construction of phylogenies, including identification of cryptic species, from DNA sequences. We ensured a period of overlap at York, so that Darwin Fellow 1 could participate in the initial training of Darwin Fellow 2. Over the same period, Darwin Fellow 2 made practical use of the training in advanced morphological identification techniques, given during the previous reporting period, to continue identification of difficult species at York and complete an inventory of butterfly species. There were two key milestones not reached: (1) UK staff were not able to make a fourth visit to Sabah in March 2003, but we were able to make use of our strong links with collaborators and support staff in Sabah to ensure that this did not create any problems for the project: (2) Educational packs were not yet in use in Sabah, partly because the local Educational Officer changed posts and was not replaced during the reporting period. This problem should hopefully be resolved during the next reporting period, when a new Education Officer is appointed.

There were no other major difficulties encountered during the year. Extraction of DNA proved more difficult than expected from one species of butterfly but with perseverance, this problem was eventually solved.

Timetable for next reporting period:

Completion date	Milestone
October	DRF 2 completes training in molecular genetics and construction of DNA-based phylogeny at York
	DRF 1 completes collection of data and biological material in Sabah
	DRF 1 & 2 complete molecular genetic analyses at Yrok
	UK staff make final visit to Sabah

5. Partnerships

Collaboration with host country partners is very strong, having developed during our previous Darwin Initiative project. During the reporting period, this collaboration has been very valuable in ensuring that our inability to visit Sabah in March 2003 did not have any adverse effects on the work of the project.

Darwin Research Fellows, Sabahan collaborators and UK staff have continued to benefit from the opportunity for discussions and cross-fertilization between different Darwin Initiative projects based in Sabah and from interactions with research scientists from a wide variety of countries carrying out projects in Sabah.

6. Impact and Sustainability

The project has a reasonably high profile, which is enhanced by its links with the Royal Society SE Asian Rainforest Research Programme, and has been further raised by our activities during our previous Darwin Initiative project and during the previous reporting period of the current project (see 1st Annual Report). In addition during the current reporting period, the project Director and Fieldwork Manager have made personal visits to senior staff at the Sabah Foundation, Universiti Malaysia Sabah and Forest Research Centre Sabah, to help maintain the profile of the project within those organizations.

7. Outputs, Outcomes and Dissemination

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

Code No.	Quantity	Description
5	2	Sabahan graduate biologists (DRF 1 & 2) received one year's training in taxonomic and molecular genetic techniques
13B	2	Labelled and annotated collections of specimens produced, to be housed at Institute of Tropcal Biology, Universiti Malaysia Sabah and Forest reseach Centre, Sabah
8	2	UK staff visited Sabah for 6 weeks each

14B	3	Results presented at conferences and seminars in Sabah and UK: included oral paper by Darwin Fellow 2 and prize to Darwin Fellow 1 for best poster at International Conservation Conference, Cambridge University

These outputs are broadly in keeping with the initial Project Implementation Timetable and the Project Outputs Schedule. One of our Sabahan collaborators (Dr Chey vun Khen) was due to spend two weeks in the UK during the reporting period, but this visit has been postponed until July 2003, to enable him to present a paper at a symposium that we have organized as part of the British Ecological Society/Association for Tropical Biology conference on Biotic Interactions in the Tropics, where some of the results of our Darwin project work will be presented to a large international audience.

Publications (asterix denotes papers submitted with report)

(1) Hamer, K.C., Hill, J.K., Benedick, S., Mustaffa, N., Sherratt, T.N., Maryati, M. & Chey, V.K. (2003). Ecology of butterflies in natural and selectively-logged forests of northern Borneo: the importance of habitat heterogeneity. *Journal of Applied Ecology* 40: 150-162*.

(2) Hill, J.K., Hamer, K.C., Dawood, M.M., Tangah, J. & Chey, V.K. (2003). Rainfall but not selective logging affects changes in abundance of a tropical forest butterfly in Sabah, Borneo. *Journal of Tropical Ecology* 19: 35-42*.

8. Project Expenditure

Table 3: Project expenditure during the reporting period

Item	Budget	Expenditure

9. Monitoring, Evaluation and Lessons

The project's main purpose is training plus data gathering and capacity building to assist conservationists, ecologists and forest managers in Sabah with promoting responsible economic growth that maximizes the development of agriculture and silviculture whilst minimizing the impacts of loss and fragmentation of rainforests on biodiversity. The effectiveness of our training is monitored through the ability of the Darwin Research Fellows independently to collect high-quality field data and specimens and to produce replicable unambiguous DNA sequence data. Both of these outcomes have been achieved during the reporting period. The effectiveness of our data in contributing to the project purpose will be assessed from our ability to use these data to provide clear practical advice.

10. Author(s) / Date

Keith Hamer / 30th June 2003

Appendix 1. Logical framework.

Project summary	Measurable indicators	Means of verification	Important assumptions
Goal To assist conservationists, ecologists and forest managers in Sabah with promoting responsible economic growth that balances maximizing agricultural development with minimizing the impacts of loss and fragmentation of rainforest on biodiversity	At end of project, guidelines on the most appropriate sizes and placements of forest fragments will be incorporated into economic planning, and molecular genetic techniques firmly established for measuring and promoting biodiversity	Reports and guidelines received and accepted by State Agencies. Trained Sabahan staff in post and using molecular genetic facilities at UMS to develop new projects.	N/A
Purpose To provide clear practical advice on the sizes and placements of forest patches necessary to preserve species richness and genetic diversity. To leave a lasting legacy of trained Sabahan staff using molecular genetic techniques to measure and promote biodiversity within Sabah and throughout SE Asia.	Practical assistance given to forest managers and conservationists (March 2004). Molecular genetic tools used to resolve otherwise intractable taxonomic uncertainties and identify cryptic species (Sep 2002). Darwin Research Fellows able to use molecular genetic and ecological techniques to develop further projects (March 2004).	Management guidelines written to assist in maintenance and promotion of biodiversity in forest fragments. Biodiversity inventories and guides updated after resolution of taxonomic difficulties.	Forest managers and conservationists have a meaningful input into economic planning. This is guaranteed by the State legislature. Molecular genetics facilities are maintained at UMS beyond the life of the project. Internal funding has already been approved at UMS, subject to availability of suitably trained staff.
Outputs Quantitative elucidation of how species diversity and genetic diversity within species relate to forest patch size and	Research papers written up (March 2004). Successful completion of training courses by Darwin Research	Research papers published in peer-reviewed scientific journals. Darwin Research Fellows attain	Darwin Research Fellows take up posts at UMS and FRC. This is almost certainly

isolation. Training of two Sabahan Darwin Research Fellows in ecological and molecular techniques for measuring species richness and quantifying genetic diversity.	Fellows (September 2002 for DRF I, September 2003 for DFR II).	appropriate standard in written, oral and practical examinations.	guaranteed by the recruitment process. Research leads to clear guidelines.
Activities Collection and analysis of data on biodiversity in selected forest fragments. Attendance by Darwin Research Fellows of training course at Durham with additional training for both Fellows in Sabah.	Fieldwork sites established (Sep 2001), material collected (Apr 2001-Sep 2003), Darwin Research Fellows attend courses and receive in-house training at Durham (Oct 2001-Sep 2002 and Oct 2002-Sep 2003).	Map of study sites produced; collection of material established; Darwin Research Fellows appointed and arrive in Durham.	Successful recruitment of Darwin Research Fellows and establishment of study plots in forest fragments. Both of these are greatly facilitated by the close links that we have with our collaborating institutions in Sabah.