



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

1st Annual report



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Front cover shows *Polyura jalysus*, a specialist of naturally occurring gaps in tropical rainforests.

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	1 st 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 liaising 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 during the reporting period. The operational plan has, however, been modified following the Project Director's move from the University of Durham to the University of Leeds. Training and analysis of molecular genetics is now taking place at the University of York, where the Project Fieldwork Manager is based, under the supervision of the project's new Molecular Genetics Manager, Dr Jeremy Searle. These changes have been approved by the Darwin Secretariat.

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.

All key milestones were reached: 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. UK staff made two visits to Sabah and work commenced on production of educational packs. 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.

The two Darwin Fellows were chosen on the basis of their exemplary performance during our previous Darwin Initiative project, because they had already received the necessary basic training in butterfly sampling and identification techniques during that project, and because they are likely to be in a strong position to move on to paid employment within biological conservation in Sabah at the end of the current project. During the reporting period, Darwin Fellow 1 received 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. Over the same period, Darwin Fellow 2 received training in advanced morphological identification techniques including interpretation and refinement of bifurcating keys, dissection and preparation of genitalia, and quantitative analysis of wing-spot pattern. During this period we also carried out a reconnaissance of potential study sites, including extensive liaison with local authorities within Sabah, as a result of which we were able to identify 12 key sites ranging in size from very small forest fragments to very large areas of continuous forest. These were all then sampled at least once during the reporting period, using standardized techniques developed during our previous Darwin Initiative project. This resulted in a good sample of suitable specimens which were transported to York under appropriate licences, to be used by Darwin Fellow 1 to construct 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).

There were no major difficulties encountered during the year. The project design has been enhanced by the inclusion of forest inventories for every forest fragment, which will permit a more sophisticated and informative analysis of differences among fragments in faunal composition and genetic diversity, and by the addition of novel sampling techniques that will allow the inclusion of a wider range of butterfly taxa and trophic categories.

Timetable for next reporting period:

Completion date	Milestone
October	DRF 1 completes training in molecular genetics and construction of DNA-based phylogeny at York
	DRF 2 completes collection of data and biological material in Sabah
	DRF 2 commences training in molecular genetics at York
	DRF 1 commences collection of data and biological material in Sabah
	UK staff make third visit to Sabah
December	DRF 1 presents data at conservation conference in Cambridge
March	Data and biological material taken to York for analysis by DRF2
	Inventory of butterfly species produced
	UK staff make fourth 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 facilitating access and permission to sample in forest fragments and instrumental in allowing us to obtain the necessary licences to export biological material from the host country.

Darwin Research Fellows, Sabahan collaborators and UK staff have all benefited 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. We have given information and educational material including 35mm transparencies and postcards bearing the Darwin Initiative logo to the Education Officer for Danum Valley Research Centre, so that the large number of school children and other members of the public visiting the Centre can be made aware of the project. Its profile was also raised at the Darwin Initiative conference held at Universiti Malaysia Sabah in September 2001.

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 censusing, taxonomic and molecular genetic techniques
6A	1	Field assistant received 10 weeks training in sampling and identification techniques
22	240	Study plots established in 12 forest fragments for longterm monitoring of butterfly biodiversity
8	2	UK staff visited Sabah for 9 weeks each
17C	1	Web page to disseminate information about Darwin Initiative projects enhanced
14B	3	Results presented at conferences and seminars in Sabah and UK

These outputs are broadly in keeping with the initial Project Implementation Timetable and the Project Outputs Schedule. The number of forest fragments is smaller than originally planned because of unforeseen difficulties in gaining safe access to some sites and because we felt it was more important to obtain replicated data from each fragment rather than to collect lower quality data from a larger number of sites.

Table 2: Publications

None during reporting period

Information about the project was presented at the Darwin Initiative conference at Universiti Malaysia Sabah, in September 2001. Posters were also produced for display at UMS. Two seminars were given at Danum Valley Research Centre, Sabah.

8. Project Expenditure

Table 3: Project expenditure during the reporting period

Item	Budget	Expenditure
Salaries (Darwin Research Fellows)		
Rent ,rates heating lighting etc		
Office administration costs		
Capital items/equipment		
Others		
Total		

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 / 5th March 2003

Appendix 1. Logical framework.

Project summary	Measurable indicators	Means of verification	Important assumptions
<p>Goal</p> <p>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</p>	<p>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</p>	<p>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.</p>	<p>N/A</p>
<p>Purpose</p> <p>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.</p>	<p>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).</p>	<p>Management guidelines written to assist in maintenance and promotion of biodiversity in forest fragments. Biodiversity inventories and guides updated after resolution of taxonomic difficulties.</p>	<p>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.</p>
<p>Outputs</p> <p>Quantitative elucidation of how</p>	<p>Research papers written up (March</p>	<p>Research papers published in peer-</p>	<p>Darwin Research Fellows take up</p>

<p>species diversity and genetic diversity within species relate to forest patch size and isolation. Training of two Sabahan Darwin Research Fellows in ecological and molecular techniques for measuring species richness and quantifying genetic diversity.</p>	<p>2004). Successful completion of training courses by Darwin Research Fellows (September 2002 for DRF I, September 2003 for DFR II).</p>	<p>reviewed scientific journals. Darwin Research Fellows attain appropriate standard in written, oral and practical examinations.</p>	<p>posts at UMS and FRC. This is almost certainly guaranteed by the recruitment process. Research leads to clear guidelines.</p>
<p>Activities</p> <p>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.</p>	<p>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).</p>	<p>Map of study sites produced; collection of material established; Darwin Research Fellows appointed and arrive in Durham.</p>	<p>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.</p>