Expedition Report For The Davis Expedition Fund

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Project Title: Genetic Variation In Two Species Of *Magnolia* In Mexico, *M. Sharpii* And *M. Schiedeana*

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Collaborators: Dr Mario Gonzales, ECOSUR, Chiapas, Mexico.

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Aims:

The **specific aim** of this research project is to assess the level and structure of genetic variation in two Mexican montane forest tree species; *Magnolia, Magnolia sharpii* and *Magnolia schiedeana*. Both species are endemic to Mexico and have been reduced to a few remnant populations, with *Magnolia sharpii* being known to occur in only five small populations in the state of Chiapas. Both species are listed as threatened with extinction. Although very little is known about these species ecology, *Magnolias* are valued internationally as ornamentals as well as being important local resources for indigenous peoples. The aim is being achieved by working through several **subaims**:

1. Locate populations of *M. sharpii* and *M. schiedeana*.
2. Obtain suitable leaf samples during a sample collecting expedition to these populations.
3. Optimise DNA extraction protocol.
4. Extract DNA from leaf samples.
5. Optimise the selected marker system protocol.
6. Carry out the selected marker system on the DNA samples.
7. Analyse data:

- Calculate the level of genetic variation and its distribution within and between populations.
- Compare the results to null hypotheses constructed from predictions based on life history traits of *M. sharpii* and *M. schiedeana*.
- Evaluate the relative influence of seed and pollen dispersal in total gene flow by contrasting the spatial distribution of the differently inherited markers.

The **overall aim** of the fieldwork, and the project as a whole, is to contribute to the development of a conservation strategy for these species by:

- Providing information on their genetic variation which can be used to evaluate the genetic implications of their conservation.
- Establishing a baseline for monitoring the processes influencing the viability of small, isolated populations and quantifying future human impacts.

Fieldwork (15th March - 5th April 1998):

Subaims 1 and 2 were fulfilled during the fieldwork in Mexico. In Chiapas, university staff at ECOSUR collaborated with me to locate and sample four populations of *M. sharpii*. In Veracruz, the staff at the Instituto de Ecologia helped in locating and sampling three populations of *M. schiedeana*. 30 individual trees from each population were sampled for leaf and bud material. Fresh and dried material was stored and brought back to the UK for laboratory analysis (for details of the laboratory work see the section below on genetic marker systems). Voucher specimens were collected from the sites and deposited in the relevant institute's herbarium when such a specimen did not previously exist. This will provide permanent, physical documentation.

In addition to the sample collecting, there were several other important advancements made during the fieldwork. The resources available at both ECOSUR and the Instituto de Ecologia proved invaluable. Their map rooms provided me with detailed information on the sites were the Magnolia populations were found. Also, their herbariums had *M. sharpii* and *M. schiedeana* specimens whose accompanying information allowed me to collate a distribution map of these species. This map shows their known range over a period of time. This can be compared to their known present day distribution, which is more restricted due to habitat destruction. In addition, the expertise of the staff and visiting academics at the collaborating institutes provided me with useful information on the species and on specific matters, such as data analysis.

Genetic Marker Systems:

Three marker systems are being investigated in the laboratory: isozymes, Restriction Fragment Length Polymorphisms (RFLPs) of chloroplast DNA PCR product and RAPDs. Isozyme analysis has been successful using fresh and frozen material. The DNA extraction protocol has been optimised for dried leaf material. The chloroplast DNA marker system has been investigated. Intraspecific cpDNA detection has been greatly facilitated by the increased resolution from PCR amplification of the fastest mutating, non coding regions. Universal primers have been designed to enable this PCR amplification over a wide range of taxa. I have successfully amplified 6 such fragments, 2 Taberlet and 4 Demesure. These fragments have been restriction digested with 8 different 4bp enzymes. Four potential polymorphisms have been found from this; three are intraspecific and one is interspecific. Single Stranded Conformation Polymorphism (SSCP) and sequencing will also be used to screen for polymorphisms.