

Dear Sir/Madam,

I would like to apply to the Davis Expedition Fund for Fieldwork with my project entitled "Evolution of *Begonia* section *Gireoudia*". I graduated in the summer of 2008 from the University of Edinburgh's School of Biological Sciences, and I am now a PhD student at the University of Edinburgh, with most of my research based at The Royal Botanic Gardens Edinburgh (RBGE).

The project I am seeking funds for is intended to aid our understanding of the factors contributing to tropical diversity, using the species-rich genus *Begonia* as a model system. We plan a collection and study trip to southern Mexico, which is a centre of diversity for *Begonia* section *Gireoudia*, one of the most evolutionarily interesting *Begonia* sections. This area is underexplored and many species are known from only one or two old collections. Many of these species may be threatened by habitat destruction. We will make herbarium, DNA and seed collections and collect habitat and morphological data from *Begonia* populations and hybrid zones. This material and data will be used to reconstruct and understand the evolutionary history of *Begonia* and to safeguard these species in *ex situ* collections.

The experience gained by tropical field work is invaluable for botanists in their careers. This field trip will greatly benefit the relations between botanical research institutes in Scotland and Mexico, and long term collaborations from this project would be of great benefit to both.

I am pleased to attached the full grant application, and look forward to hearing the decision of the committee.

Yours sincerely,

The Evolution of *Begonia* section *Gireoudia*

An assessment of species diversity, gene flow and
hybridization in Mexican Begonias



**By the Royal Botanic Gardens Edinburgh
&
The University of Edinburgh**

Introduction

Tropical regions harbour much of the earth's biodiversity. Yet the manner in which this diversity has arisen and is maintained remains unclear. To shed light on the factors contributing to this diversity, *Begonia* is an excellent a model system to understand how tropical diversity has evolved and is maintained. *Begonia* is one of the largest angiosperm genera (>1500 species), populations of which are often, but not always, small and isolated. *Begonia* species exhibit a very wide range of morphological variation, particularly in leaf shape. Much of this variation appears to be adaptive and to have evolved repeatedly in different lineages within the genus. *Begonia* is therefore an ideal group for examining the relative contributions to speciation of ecological differentiation and genetic isolation. Furthermore, multiple species of *Begonia* often co-occur. On some occasions they produce hybrids, yet the species still maintain their distinctive identities. Therefore, *Begonia* is also an ideal group for investigating the coexistence of multiple closely related species in the tropics.

At Royal Botanic Garden, Edinburgh, we have begun a research project to understand the evolution of diversity in *Begonia* Section Gireoudia, a monophyletic group of about 66 Central American *Begonia* species. The group is notable for its high morphological diversity and some morphological characters appear to have arisen on multiple occasions in different lineages, making Section Gireoudia an excellent group for studying the developmental genetic basis of morphological differences between *Begonia* species. Some of these morphological differences may be related to distinct habitat variables - *Begonia* Section Gireoudia is known from an especially wide range of habitats from dry, desert scrub through to wet rainforest, and at altitudes from sea level to over 3000 meters (Burt-Utley, 1985). However, as with *Begonia* in general, Section Gireoudia is poorly known in the wild even at the species level and there is little detailed knowledge of the habitats of the species and their population structure. For many species, only one or very few individuals formed the basis of the original material brought into cultivation, and many of the species are not represented at all in UK collections or elsewhere. Due to the preparation methods for herbarium samples in the tropics, no DNA is available from type specimens to compare with these cultivated accessions.

Therefore, to address the broader evolutionary questions in *Begonia* Section Gireoudia, we require documented DNA and seed collections of the plant populations and their hybrid zones from their natural habitats, together with data on species ecological requirements, population sizes and distributions. Furthermore, many of the species are thought to be narrow endemics and are known only from one or two localities. This is especially so for the underexplored Mexican species. Some of these,

such as the very distinctive *B. carrieae*, have not been observed in the wild since their original collection. Many of the forest habitats of these species are currently under considerable threat from habitat modification and the status of the *Begonia* species is unknown.

My PhD is focused on understanding hybridisation in *Begonia* Section *Gireoudia*. As generalist-pollinated species, *Begonias* do not appear to have strong barriers against interspecific hybridisation and hybrids are commonly observed in nature, yet they appear to maintain their specific identities; they thus provide a good system for the study of reproductive isolation. On the other hand, gene exchange (introgression) could also be a factor in the convergent evolution seen in this group. Hybridisation appears to be historically important in the group, and has been implicated in the initial invasion of South America by *Begonias* (Goodall-Copestake 2006).

Several *Begonia* Section *Gireoudia* species have overlapping distributions where hybrids are occasionally found (Burt-Utley 1985, McMillan et al., 2006). Field observations by McMillan et al. (2006) identified putative hybrids between *Begonia nelumbiifolia* and *B. heracleifolia*. Our morphological data on greenhouse created F1s and F1BC1 suggest this was in fact an F1 backcross plant. Multigenerational hybridisation, implying possible gene exchange, could be occurring at this site and at a number of other locations where hybridisation has been observed in the wild. Population genetic studies of wild collected material, using morphological data and microsatellite markers, would help us understand the degree of introgression within populations.

Together with a colleague from RBGE, I am planning a 5-week collecting and study expedition to southern Mexico to collect herbarium, DNA and seed material from *Begonia* Section *Gireoudia* populations, to make detailed observations of plant populations and habitats, and to collect from, and morphologically characterise, natural hybrid zones. The southern Mexican states of Chiapas, Oaxaca and Veracruz form the most important centre of diversity for *Begonia* Section *Gireoudia*, and showcase the full range of morphological and habitat diversity of the group. Of these states, species distributions have been described for Veracruz (McMillan et al. 2006), where a number of hybrid zones have been noted. However, Oaxaca and Chiapas remain poorly known and are likely to yield novel discoveries or rediscoveries of "lost" species.

I plan to visit several of the potential hybrid zones previously identified (McMillan et al., 2006, Burt-Utley 1985) and sample putative hybrids, parents and neighboring populations to determine if introgression is occurring. I will especially focus on species-pairs which are known to form hybrid zones in multiple locations and which are the focus of my greenhouse-based work at RBGE. Initial genotyping of this material will be by restriction analysis of chloroplast DNA on return to RBGE. This material will form the basis for more extensive molecular characterisation of hybrid zones in this *Begonia*

Section *Gireoudia* at RBGE. I will also use morphometrics to characterise the hybrids and their putative parental populations. This will determine the extent of hybridisation and introgression occurring in the wild, allowing me to formulate hypothesis about their roles in speciation in this highly speciose genus.

This project will provide me with an invaluable experience of tropical botanical fieldwork which would not otherwise be available to me, and which will make a significant contribution to my scientific education. In addition, the opportunity of seeing *Begonias* in their natural environment will provide a broader context to my PhD studies on *Begonia* in the laboratory. As preparation for this project, I have become familiar with many of the species in the section, as well as the phenotypes of F1 progeny from greenhouse crosses. It would be beneficial for me to see hybrids and species in the field, to compare these to those maintained in the greenhouse, and observe their natural habitat. I have previously worked in the field in the UK and continental Europe but working in the tropics would be a valuable addition to my botanical education.

References

Burt-Utley, K. 1985 *Tulane Studies in Botany and Zoology* 25:3-123.

Goodall-Copestake 2006. PhD thesis University of Glasgow.

McMillan, P.D., Wyatt, G. and Morris, R. 2006. *Acta Botanica Mexicana* 75:77-99.

Project outline and aims

Together with another colleague from RBGE, Dr.*, and a Mexican colleague, I have planned a field trip to collect from populations of 30-40 species of *Begonia* Section *Gireoudia*, with a focus on the most underexplored but species-rich areas of southern Mexico.



We plan to carry out 5 weeks of field work in March 2010, when the plants are flowering and fruiting. For the rare and endemic species, we will return as close as possible to the original collection localities, in an attempt to re-find these species in the wild, as well as explore likely habitats in the surrounding areas, most of which are quite remote and botanically underexplored in general. It is likely that we will find a number of novel forms, and possibly species, during these explorations. In addition, we will collect plants from throughout the range of widespread species to represent the full diversity within each species, and will study and collect from previously described natural hybrid zones. Overall, the aims of the project are:

(a) collect DNA samples and herbarium specimens from populations of the majority of species of *Begonia* Section *Gireoudia* in Southern Mexico (30-40 species), by visiting locations of known high species diversity. The DNA material will be used to construct a robust phylogeny for the group and for future phylogeographic work. A complete digital photographic record will be made of the plants and the habitat they grow in.

(b) collect seed from as many species as possible to be grown up at RBGE and Glasgow Botanic Garden for future developmental and evolutionary genetic investigations and to serve as a seed bank for rare or threatened species. These

Plants will be grown up and maintained at RBGE and in duplicate at Glasgow Botanic Garden. These new accessions will significantly enhance the *Begonia* reference collections at both these institutions.

(c) undertake basic ecological descriptive measurements to gain initial insight into the role of niche differences and adaptation in *Begonia* speciation. This data will be supplemented with edaphic, geological and climate data on the collection locations.

(d) assess the population sizes of all *Begonia* Section Gireoudia populations and hence their conservation status.

(e) collect samples of 30 individuals along a transect for future population genetics analyses of a focal set of three widespread and three range-restricted species from different habitat types.

(f) conduct an investigation of the role of hybridization in *Begonia* Section Gireoudia by investigating 5-6 natural hybrid zones in detail, including morphological characterisation of hybrids and their parents, and collection of seeds and DNA material of both hybrids and surrounding parental populations.

Participants

*

PhD student, The University of Edinburgh and The Royal Botanic Gardens Edinburgh.

My previous field work experiences include attending field courses as an undergraduate, as well as teaching on field courses. Most recently, I taught on the University of Edinburgh Plant Sciences Field trip to the Burren, Ireland. My responsibilities on this course was overseeing student projects, and helping with plant identification. In 2007 my field report on the ecology of orchid pollination in Italy won the university's Dobby Smith Prize.

I am experienced at plant identification, and demonstrate on Edinburgh University's Evolution and Ecology of Plants and Origin and Diversity of Life courses. I am a keen photographer, and I have given a presentation on macro photography to colleagues at RBGE.

Dr *

Senior Research Associate, The University of Edinburgh and The Royal Botanic Gardens Edinburgh.

* is an experienced botanist, who has previously done considerable botanical fieldwork in Mexico, as well as in many other countries, and is a good Spanish speaker. He is currently working as part of the *Begonia* group at RBGE.

Dr. * is an investigator from the Instituto de Biología, Universidad Autónoma de México (UNAM) and a long-term collaborator with colleagues at RBGE. He is assisting us with the permit application process and arranging for a colleague from UNAM to accompany us in the field (this is an essential requirement for all field work in Mexico).

Project Outcomes

This research will form part of my Ph.D. dissertation and I also plan to publish the results in high-impact peer-reviewed journals on the following topics:

- The role of ecological selection in the maintenance of species barriers in *Begonia*: a comparison of introgression in lab crosses versus natural hybrid zones.
- The role of gene flow in *Begonia* evolution.

I also plan to disseminate my results at an international conference and locally both at Edinburgh University and to the Botanical Society of Scotland. Additional outputs include:

- Fully sourced seed and DNA material for future evolutionary genetic and hybridisation investigations in *Begonia* Section *Gireoudia*, and, in the case of seed, an *ex situ* conservation resource for rare species.
- Conservation assessment of species in *Begonia* Section *Gireoudia*.
- Fully documented herbarium samples deposited in RBGE and UNAM (Mexico) herbaria.
- Collaborative ties with Mexican and Costa Rican botanists.

Schedule

28 th Feb:	Fly to Mexico.
1-2 March:	Planning, acclimation in Mexico City
3-7 March:	Vera Cruz – Orizaba-Cordoba region.
8-12 March:	Vera Cruz – Los Tuxtlas.
13-19 March:	Oaxaca – Sierra Juarez.
20-28 March:	Chiapas – Sierra Madre de Chiapas
29-30 March:	Chiapas – around San Cristobal de las Casas
31 March-4 April :	Chiapas – Ocozocuatla region
5 April:	Return to Mexico City
6 April:	Mexico City – sort specimens
7 April:	Return to Edinburgh

Technical Issues

Permits

Collection permits are required and in Mexico, all foreign collectors must also be accompanied in their collection trips by a Mexican Botanist. Arrangements for this are being made with Dr Alfonso Delgado Salinas from UNAM in Mexico, who has previously collaborated with RBGE.

Health and safety

We envision few problems with the collection trip, other than the usual hazards of driving on poor roads in mountainous terrain. The nominated driver, *, has extensive field work experience in such conditions in Mexico and all suitable precautions will be taken. In addition, we will be accompanied at all times by an experienced Mexican field botanist.

Funding

The total cost to fund for the fieldtrip is £6638. I would like to apply to the Davis Expedition Fund for £2500.

Overall, the total cost breakdown is as follows:

Air fare £1298

Buses/trains £240

Accommodation £1600*

Food £1720*

Insurance £188

Visas and permits £75 (for 2)

Hire car (including petrol) £1295

Silica and maps £145

* includes Mexican colleague.

Applications made

Percey Sladen Memorial Fund	£750	Successful (see below)
Weir Fund for Fieldwork	£1200	Successful (see below)
Royal Horticultural Society Bursary	£2000	Decision pending
Merlin Trust	£750	Decision pending
Rennie Fund	*	Unsuccessful
Edinburgh Small Grants	Joint application	Unsuccessful
Stanley Smith Horticultural Trust *		Unsuccessful

The Linnean Society and Weir Fund were successful for a proposed 2009 expedition, but due to the late start for grant applications insufficient time was available to obtain permits. Applications to transfer this funding to 2010 have been made.



21st January 2009

Reference for *
Re. Davis application

I have known * for several years, first as an undergraduate when he studied Ecology and Evolution of Plants, and Angiosperm evolution, then as a PhD student. Academically he is very able and narrowly missed a first due to a single poor result in a third year course. He was a very keen student and participated in all the class discussions and produced written work of exceptional standard.

He was interested in gaining lab experience and applied for funds from Nuffield and the Genetics Society to work in my lab during the summer of 2007 where he studied variation in anthocyanins in *Begonia* using molecular and physiological methods. He set up collaborations with the * lab to isolate anthocyanins and the * lab to study transmission and reflection of light from leaves of different species. He also cloned several genes from different *Begonia* species using degenerate PCR. He demonstrated an exceptional ability to work well with people and a very practical approach to problems.

His undergraduate thesis was also based at RBGE, this time working with * on *Vireya* *Rhododendron* biogeography. For this work he taught himself phylogenetic dating techniques and he was invited to present the results in at the British Ecological Society's 2008 meeting. Throughout this time he has been involved in outreach activities such as the Press Gang and is currently Researcher in Residence at Trinity School

Alex was awarded a BBRSC PhD studentship and started his PhD on hybrid vigour and dysgenesis in *Begonia* in September 2008. He will be analysing phylogenetic and geographic patterns of hybrid vigour and fertility and using our mapping population to QTL map fertility in interspecific crosses. He is an excellent student and great fun to work with. He has made a very good start analysing growth rates and fertility in the interspecific and backcross hybrids we have created and is working on a collaboration with a commercial *Begonia* breeding company in Holland to exchange data and screen populations. The proposed fieldwork will greatly expand the possibilities of his PhD work, allowing him to analysis hybrid populations in the wild and expand the number of accessions he will be able to work with. The experience of working with the plants in the wild will give him insights into their biology invaluable for interpretation of the data he is gathering in the greenhouse. It will also give him valuable experience in tropical field botany and strength links between Mexico and RBGE, hopefully leading to further collaboration and field experiments.

I strongly support this application,

Yours sincerely,