#### **Darwin Initiative for the Survival of Species**

#### **Annual Report**

# 1. Darwin Project Information

Project Ref. Number	162/12/008		
Project Title	DNA banking, phylogeny and conservation of the South		
	African flora		
Country(ies)	South Africa		
UK Contractor	Royal Botanic Gardens, Kew (RBG Kew)		
Partner Organisation(s)	National Botanical Institute (NBI)		
	University of Cape Town (UCT)		
	University of Stellenbosch (US)		
	Rand Afrikaans University (RAU)		
Darwin Grant Value	£116,187		
Start/End dates	01/06/2003 — 31/03/2006		
Reporting period (1 Apr 200x	01/06/2003 — 31/03/2004		
to 31 Mar 200y) and report number (1,2,3)	Report 1		
Project website	http://www.nbi.ac.za/research/dnabank.htm		
Author(s), date	Dr Vincent Savolainen (RBG Kew, UK) & Dr Gail Reeves		
	(NBI, South Africa) 16/04/2004		

#### 2. Project Background

South Africa possesses a unique flora with two global biodiversity hotspots within its borders. The wealth of botanical knowledge and expertise in South Africa is reflected in its extensive herbarium collections, housed by both the NBI and academic institutions. However, there is a pressing need for South Africa to take control of its genetic resources, and to utilize these accordingly. DNA banking represents one such avenue to address these short-comings, and therefore this project has enabled the financial input, in addition to skills and knowledge transfer, required to establish a truly modern botanical facility for high-profile research and conservation in South Africa.

# 3. Project Purpose and Outputs

The project aims to (i) archive in a DNA Bank the genetic material of at least one species from nearly all ca. 2200 South African flowering plant genera, of which 70% spp. are endemic; (ii) provide the necessary facility to extract DNA and preserve it appropriately, and to allow researchers to have access to plant DNA extracts to be used in applied and fundamental science; (iii) through collaboration with RBG Kew's CBD Unit which has considerable experience in the topic, to implement the necessary legal agreements for material transfer and benefit sharing of these genetic resources; (iv) train South African researchers and students, including those from disadvantaged communities, in high-profile biotechnologies; (v) produce a phylogenetic 'tree of life' of South African plant genera and identify areas of endemicity and high priority for the conservation of biodiversity. This phylogenetic information will also enable more focused bioprospecting strategies to be adopted by the academic sector in their search for natural plant products (including those that are medicinally important. The outputs or proposed operational plan have not been modified over the last year.

#### 4. Progress

This project was initiated in June 2003, following a request from DEFRA that the project begins two months later than originally planned. This is the first reporting period and therefore we have no information to provide with regard to the history of the project prior to the beginning of this reporting period.

# 4.1. Summary progress

Since commencement of the project in June 2003 a plant DNA bank has now been established in South Africa. Progress has been monitored according to four main activities: 1) Workshops, 2) Fieldwork and laboratory research, 3) Courses, lectures and practicals and 4) Manual, publications and publicity. Summary progress over the last year against the logical framework is provided in Annex 1.

# 4.1.1.Workshops

From July 1-3rd 2003 a project planning workshop was hosted by the Royal Botanic Gardens Kew (RBG Kew), attended by 15 participants from the UK and four from South Africa. During this time a MoU was written and subsequently signed by both parties (Annex 2). Research presentations were given by participants from both countries and the existing DNA banking facility at RBG Kew was visited by the South Africans. Financial arrangements were also established during the course of this workshop, and an outline for the 'DNA banking manual' was drawn up. From 3-5th September 2003 the second workshop was hosted by the NBI at Kirstenbosch in South Africa. This was attended by two representatives from RBG Kew, and 27 South African scientists took part in the 'Information and Feedback' component. Dr Vincent Savolainen presented the DNA bank setup at Kew to the South African participants, and prompted considerable discussion regarding the use and potential misuse of such a facility. Dr Maureen Wolfsen (NBI) presented the South African perspective relating to equitable use and benefit sharing of genetic resources. During the course of this workshop it was also agreed that Ms Ingrid Nanni (NBI) will act as co-administrator to the project in South Africa. Ms Nanni has considerable project-management experience (she has been project manager for the Conservation Farming project for the last three years at the NBI funded by the World Bank). This move has further demonstrated the NBI's commitment to this project. Ms Nanni's main functions are to liaise with HR and finance at NBI, and with South African authorities regarding the establishment of a country-wide collecting program for the DNA bank. Through the workshops the following members of NBI staff have been trained in the management of genetic resources: G. Reeves, K. Balele, F. Conrad, I. Nanni and M. Wolfson. From Kew, V. Savolainen, C. Williams, K. Davis, and M. Chase have also gained knowledge concerning the establishment of a DNA banking facility in partnership with a developing country. University (L. Dreyer, K. Oberlander and D. Bellstedt) and conservation authority (A. Le Roux) representatives also participated in the workshop held in South Africa.

#### 4.1.2. Fieldwork and laboratory research

Collection of material (leaf samples for DNA extraction and associated voucher specimens for the Compton Herbarium at NBI) was initiated and a total of 473 species were collected during this reporting period. The number of South African genera collected during this time period was considerably less than was projected in the project proposal, with only 149 out of an anticipated 1000 genera sampled. Two extenuating circumstances have led to this shortfall. Firstly, severe drought during the spring (August-October) of 2003 led to the poorest flowering season witnessed in the Cape Region in the last twenty years. This impacted heavily on our ability to collect as many samples as we originally anticipated. Secondly, mobilisation and volunteering of the botanical community towards collecting plants in this poor flowering season has been very limited and only Kew and NBI staff have so far collected for the bank. Therefore a comprehensive collecting programme was not set up during this reporting period.

In August 2003 capital equipment was purchased by the NBI and the DNA bank was formally established. Ms Kholiwe Balele was appointed to the post of DNA bank manager by the Darwin Initiative project at the NBI on 1st November 2003. As outlined in the original proposal, this appointment was made in accordance with the NBI's Affirmative Action policy,

aimed at recruiting new staff members from previously disadvantaged backgrounds (in this case a black female was appointed). Kholiwe has subsequently spent one month training at RBG Kew under the direct mentorship of Mr L. Csiba, one of Kew's DNA bank managers. During this time Kholiwe processed 1363 DNA extracts of South African plants held in Kew's DNA bank, and duplicated them to the new DNA banking facility at the NBI. This has taken the total number of South African samples now archived in the NBI bank to 2891 representing 443 genera and 2129 species (293 species previously held at NBI, 473 species/149 genera collected since June 2003, 1363 species/257 genera duplicated from Kew; see Annex 3). These DNAs are available for supply through direct request to the Leslie Hill Molecular Systematics Laboratory but a more effective web-based request process is being set up. Material transfer was extensively discussed at the Kew meeting and all agreements (GMTAs) have been included in the MoU and are running accordingly (Annex 2).

The production of a gene matrix (*rbcL* DNA sequences) aimed at producing a Tree-of-Life of South African plants for conservation and bioprospecting has also been initiated, although *rbcL* sequencing was initially targeted only for September 2004. A total of 201 *rbcL* sequences have been retrieved from GenBank/EBI in September 2003 and an additional 202 species representing genera found in South Africa were downloaded, although these latter are not species from South Africa. All these DNA sequences are still available from GenBank/EBI.

#### 4.1.3. Courses, lectures & practicals

A week practical training course on 'Biotechnology and Molecular Techniques' (28 July - 1 August 2003) was successfully completed at the NBI by 11 students from the University of the Western Cape. Another week practical course on the same topic was attended by 15 students (eight from RAU, two from US, four from UCT one from UWC) from 23 – 26 March 2004. This has exceeded our target of 12 undergraduates for this reporting period.

Three PhD students are now routinely benefiting from the DNA bank facility in place in South Africa. Ms Ferozah Conrad and Mr Christopher Cupido are registered for PhDs at UCT but based at Kirstenbosch; Mr Kenneth Oberlander is registered at US. Ferozah's PhD study on the molecular systematics of the Amaryllidaceae tribe Haemantheae also involves a population genetic study of the horticulturally important genus *Clivia*. The DNA bank has enabled her to purify the DNA extracts necessary for her to complete this project in a way that they can be stored indefinitely and accessed repeatedly. Similarly Christopher and Kenneth work on species-level phylogenetic reconstruction of South African Campanulaceae and Oxalis respectively. It is expected that F. Conrad and K. Oberlander will have completed their PhD studies by the end of this DI project.

Another eight students are registered for Hons/MSc degrees. Ms Amelia Mabunda and Ms Leanne Mannie are registered at UWC and UCT, respectively, but have not yet formalised their practical research. Mr Paul Naude, Ms Collette Robinson, Ms Cynthia Motsi, Ms Annemari van Niekerk, Ms Jerminah Matlou and Ms Maline Rautenbach are registered at RAU and work on molecular phylogenetics of Vitaceae, Thymaeleaceae, Rafnia, Passerina, Corchorus, and Gnidia respectively.

As mentioned in the previous section, Kholiwe Balele received one months training at Kew according to the terms of the technology transfer aspect of the MoU. During this training period she was exposed to all aspects of DNA extraction and storage, in particular the use of the ultracentrifuge (this is a specialised and costly item funded by DI at the NBI that is used for the purification of DNA and its long-term storage). Kholiwe acquired important skills during her visit that will enable her to perform her role as DNA bank manager in South Africa to the best of her ability. Key contacts were also established between Kholiwe and Kew's DNA bank managers. The extremely positive relationship that Kholiwe built up with her colleagues at Kew will undoubtedly play a vital role in the success of this project now and in the future.

#### 4.1.4. Manual, publications and publicity

A chapter outline has been drawn up for the DNA banking manual, and three of the proposed chapters (entitled The CBD, Implementation and Biotechnology Protocols) have been drafted during this reporting period.

In terms of the proposed field guide, this was completed well ahead of schedule with the publication of 'A photographic guide to the wildflowers of South Africa' by J. Manning in

December 2003. This was made possible due to a considerable amount of work by J. Manning being completed before the official start of this project in June 2003. The DI scheduled some funds to cover the publication of this book for 2004/05 that were accommodated by the NBI during the financial year 2003/04.

One peer-reviewed publication entitled 'A plea for DNA banking' has been submitted by V. Savolainen and G. Reeves to *Science*. The letter is currently under review in this top-rated scientific journal (submission number 42657).

G. Reeves organised the Molecular Phylogenetics sessions of the Southern Connection Conference in Cape Town (19-24)January 2004. http://web.uct.ac.za/conferences/sc2004/) and V. Savolainen presented a paper on plant (iris family) diversification in South Africa. The Southern Connection conference attracts a large number of scientists from all continents who study all aspects of biology and earth history of the Southern Continents. The theme for 2004 was 'Towards a Southern Perspective'. The conference was very successful and 328 delegates attended from 18 countries; 156 papers were presented during the week. The conference also provided the opportunity to discuss further avenues of the DI project. A visit from Kenneth Oberlander (PhD student supervised by Dr L. Dreyer, DI partner at US) to Kew in July 2004 was prepared during the conference for data analysis; Kenneth also wishes to come to Kew for a post-doc and the details of this visit are currently under discussion with V. Savolainen.

The DNA bank DI project has been publicised in various media. An item was published in Kew Scientist in October 2003 (issue 24, p. 5); this is an international newsletter reporting on news from the Living Collections, the Herbarium and the Laboratories at Kew and Wakehurst Place, available online at www.kew.org/kewscientist. Two items were also published in the National Botanical Institute News - News from the Gardens, Herbaria, Research Units and Education Centres of the NBI: 'Leslie Hill Laboratory hosts UWC students' (September 2003, p.13) and 'UK Darwin Initiative fund a DNA bank of South African Plants' (March 2004, p. 11).

A project website was also designed, see 'DNA bank' at www.nbi.ac.za/frames/researchfram.html. Documents (e.g. list of DNA extracts held in the NBI DNA bank) have been posted on the website and can be downloaded by scientists and the public for direct enquiries to the Leslie Hill laboratory. These documents on the website are also used to assist in monitoring of the project, although monthly email and phone calls between G. Reeves and V. Savolainen have been crucial in order to reach our targets.

#### 4.2. Difficulties and future work plan

As detailed above the aim of collecting 1000 genera for the DNA bank during the first year was not attained in full. This was in large part due to the lack of a coordinated collection program being in place. We are confident that this considerable shortfall will be made up during the next 12 months through the following improvements in the project setup. Firstly, Ms I. Nanni (NBI staff member and now co-project manager) and Dr. F. Forest (recipient of the Smut's Memorial Botanical Fellowship in South Africa) have been charged with the responsibility of contacting and making arrangements with botanists throughout the country to collect specifically for this project. This will be aided significantly by our ability to provide financial assistance through the project in this financial year. Secondly, promotion of the project at a country-wide level (through presentation to Working Group 1) will enable increased participation by Nature Conservation authorities in South Africa, including the formalisation of permitting agreements at the provincial level.

May 2004: Presentation at Working Group 1 by G. Reeves; Collectors contacted by I. Nanni, F. Forest and G. Reeves and permit applications submitted to conservation authorities

June 2004: One press release in South Africa.

July 2004: South African plants meeting in Zurich to be attended by G. Reeves, M. van der Bank, L. Dreyer, J. Hawkins, V. Savolainen and other delegates (http://www.systbot.unizh.ch/capeflora/). One-week training course. Second draft of manual completed. Visit from K. Oberlander to Kew.

September 2004: workshop in South Africa 'beyond a DNA bank' and joint collecting trip. 200 *rbcL* sequenced produced. At least 500 genera collected (target 1000), DNA extracted and processed in the DNA bank. New website launched.

January 2005: V. Savolainen project visit to South Africa.

March 2005: G. Reeves project visit to Kew. One-week training course. One scientific paper submitted. An additional 200 *rbcL* sequenced produced. At least a further 250 genera collected (target 500), DNA extracted and processed in the DNA bank.

# 5. Actions taken in response to previous reviews (if applicable)

Not applicable

#### 6. Partnerships

The collaboration between RBG Kew and the NBI has been extremely positive over the last year, and the relationship between the two institutions has thus far been extremely advantageous with no unforeseen difficulties or problems.

At the start of the project an MoU was agreed by both parties that detailed the expectations and obligations of both institutions. South Africa has seen immediate benefit from this Darwin Initiative project on several fronts. Already aliquots of some 1300 DNA extracts of South African plants already housed in Kew's existing DNA bank have been provided by Kew, and under the terms of the MoU, requests received by Kew for South African DNA extracts for research purposes are now referred to the South African DNA bank. Similarly all DNA extracts housed in the South African bank will be duplicated at Kew.

Both institutions have also benefited from the sharing of expertise in relation to issues of access and benefit sharing, specifically in the drafting of the relevant chapters for the DNA banking manual. Kholiwe Balele, the NBI's DNA bank manager hired by the project, has also benefited tremendously from the time she was able to spend under the mentorship of Kew's DNA bank managers during her one-month visit. In more general terms, this project has further strengthened the relationship between RBG Kew and the NBI at the highest management levels. This proven ability to work in partnership has therefore led to exploration of further initiatives to enable future collaboration. For example, Felix Forest who has recently completed his PhD (RBG Kew & Reading University) successfully applied for the Smut's Memorial Botanical Fellowship at UCT, to work on the 'Tree-of -life' of South African genera as part of this Darwin Initiative project. In addition, the NBI has been included as a partner organization in a UK led Consortium (including RBG Kew) seeking funds from the Natural Environment Research Council for a project entitled 'Measuring the state of nature; efficient assessment of the rate of biodiversity loss'. DI partners V. Savolainen, G. Reeves, T. Hedderson, L. Dreyer and M. Van der Bank from South Africa are also working in collaboration with the SABIGG (South African Big Genera) consortium, an academic interchange network grant funded by the Leverhulme Trust to J. Hawkins (DI partner at Reading), to investigate species-level evolutionary patterns and processes using DNA based phylogenies. This latter initiative will benefit tremendously from the DNA bank now in place in South Africa due to the ability to distribute DNA extracts between researchers according to the GMTAs now in place in South Africa.

#### 7. Impact and Sustainability

The profile of the project within the academic and nature conservation community in the Western Cape is high, largely due to the geographical proximity of the NBI project leaders to individuals in this region.

Significant effort was made during this reporting period for G. Reeves to make a presentation on the new DNA banking facility to a quarterly meeting of the heads of provincial Nature Conservation authorities (Working Group 1). However, due to circumstances beyond our control this was not possible during this reporting period, but this presentation has been scheduled for May 2004. Nevertheless, both Nature Conservation agencies and the academic community agree that the NBI is the natural home for a national DNA banking facility and have given their full support to this initiative. This has been reflected in material being sent for extraction by the Western Cape Nature Conservation Board, and DNA extracts being donated

to this central DNA bank facility by T. Hedderson (UCT), L. Dreyer (US) and M. van der Bank (RAU).

This project has also been promoted extensively through the Leslie Hill Molecular Systematic Laboratories training and development program. As such, 26 graduates and postgraduates have attended a one-week practical training course on 'biotechnology and molecular techniques'. Directly as a result of their participation in these training courses, one graduate (Kholiwe Balele) has gone on to be appointed by the NBI (funded by the Darwin Initiative) as DNA bank manager, and two further graduates (Amelia Mabunda and Leanne Mannie) have gone on to receive NBI MSc bursaries to continue their studies in biodiversity and conservation.

# 8. Post-Project Follow up Activities (max 300 words)

Not applicable since this is the first year report

# 9. Outputs, Outcomes and Dissemination

All targets have been met or exceeded as detailed in Table 1. We have been particularly successful in training more students during this reporting period than originally planned (26 students; target 12+2; plus 8 Msc students have registered). Additional funding has also been raised by collaborators in this project. Dissemination of information was also successful, with three press releases published and two workshops organised.

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

Code No.	Quantity	Description		
4A	10+7=17	Number of undergraduates receiving training in biotechnology at DNA bank/NBI		
4B	1 per student	One week training per student, total 17 weeks		
4C	9+8Msc projects=17	Number of postgraduates receiving training in biotechnology at DNA bank/NBI		
4D	1 per student	One week training per student, total 17 weeks, plus Msc projects ongoing		
5	1	DNA bank manager hired and trained since November 2003		
6A	9	Nine staff to set MoU/MTA at workshops on management of genetic resources		
6B	1	Two 3-days workshops		
7	1	Kew DNA extraction protocols used at NBI		
8	3 (total 5)	Savolainen & Powell 2 weeks in September 2003, Savolainen 1 week in January 2004		
10	1	'A photographic guide to the wildflowers of South Africa' published December 2003		
11B	1	'A plea for DNA banking' submitted to Science		
12A	1	DNA bank computing database established at NBI (see www.nbi.ac.za/frames/researchfram.htm)		
13A	1	DNA bank established and DNA extracts archived and species-referenced		
13B	1	Voucher specimens for DNA extracts deposited at Compton Herbarium, Cape Town		
14A	2	2 Workshops organised (July and September 2003)		

162/12/008			
14B	1	Southern Connection Conference attended by Savolainen & Reeves in January 2004	
15C	1	Item in Kew Scientist in October 2003	
15B	2	Items in NBI News	
17B	1	SABIGG network enhanced with additional funding secured from the Leverhulme Trust, UK	
20	34,807	Ultracentrifuge and related computer	
21	1	DNA bank facility established	
23	114,000	Leverhulme-funded academic interchange network grant (UK) on South African plants (£107,020) and Smut's Memorial Botanical Fellowship (South Africa) to F. Forest (80,000 ZAR)	

**Table 2: Publications** 

Type *	Detail	Publishers	Available from	Cost £
(e.g. journals, manual, CDs)	(title, author, year)	(name, city)	(e.g. contact address, website)	
Field guide*	to the wildflowers of		Briza, PO Box 56569, Arcadia 007, Pretoria, South Africa www.briza.co.za	22

# 10. Project Expenditure

Table 3: Project expenditure during the reporting period (Defra Financial Year 01 April to 31 March)

Item Budget (please indicate which document you refer to if other than your project schedule)	Expenditure Balance
---	---------------------

## 11. Monitoring, Evaluation and Lessons

V. Savolainen, UK project coordinator, completed a two-day course during this reporting period on the logical-framework approach to monitoring and evaluating projects. This has subsequently been applied to this project. The information required to monitor the project against the planned activities and target outputs, was gathered on a monthly basis via email and telephone contact between G. Reeves and V. Savolainen. For further 'live' monitoring and evaluation the project website requires improvement. G. Reeves will liase with IT staff at NBI and RBG Kew during May in order for a new version of the project website to be launched in June.

## 12. Outstanding achievements during the reporting period

We see two main achievements during this reporting period. Firstly, in only eight months a comprehensive in-country centralised facility for plant species DNA extraction and storage has been established in one of the world's most floristically diverse hotspots. This has included purchase and operation of specialised equipment, the recruitment and training of a DNA bank manager in South Africa, and duplication of over 1300 DNA extracts housed at RBG Kew to South Africa (under the terms of the MoU agreed by RBG Kew and the National Botanical Institute). As a result of these activities South Africa has the first plant DNA bank of its kind in the southern hemisphere, and certainly the first in a developing country. Secondly, the project has provided a unique opportunity for training and technology transfer. In particular, the one-week short courses in biotechnology and molecular techniques has helped to identify promising young scientists, and subsequently one was successful in applying for the position of DNA bank manager and two others have become recipients of NBI MSc bursaries (all three are black females). This is particularly relevant in our efforts to open up opportunities in biodiversity and conservation science to a broader section of society in post-apartheid South Africa.

Annex 1 Report of progress and achievements against Logical Framework for Financial Year: 2003/2004

Project summary	Measurable Indicators	Progress and Achievements April 2003-Mar 2004	Actions required/planned for next period
<ul> <li>achieve</li> <li>The conservation of biological div</li> <li>The sustainable use of its component</li> </ul>	ersity,		Continue collaborative work between Kew and NBI and enhance participation from other partner institutions in the UK (U Reading) and in South Africa (RAU, US, UCT). Publicise DNA bank more pro-actively to conservation authorities outside Western Cape. Improve collecting programme and delegate responsibility and organisation to I. Nanni from NBI. Supervise postdoc F. Forest in the use of phylogenetics for conservation at NBI. Facilitate requests for DNAs by improving project website.
Output 1 Five partner organisations able to initiate, participate in and monitor research on plant genetic resources, for application towards in situ and ex situ conservation goals and sustainable use	A minimum of 9 staff trained in biotechnologies and managment of genetic resources, in collaboration with local conservation authorities and universities	Nine staff took part in setting up MoU/MTAs and management of genetic resources in collaboration with Western Cape Nature Conservation Board (A. Le Roux) and universities (L. Dreyer, K. Oberlander and D. Bellstedt). New collaborations and funding applications (Leverhulmefunded network on South African plants, Smut's Memorial Botanical Fellowship) have been successful as part of this project	Continue collaborative work, in particular towards improving cost effective measures of biodiversity (including genetic diversity) to meet the 2010 target of decreasing the rate of loss of biodiversity as agreed at the 2002 CBD World Summit in Johannesburg
Output 2 DNA from all S African plant genera preserved for future and genes sequenced	DNAs available for supply and gene sequences deposited in GenBank/EBI	1528 DNA samples of South African plants archived and available from NBI, 403 rbcL gene sequences of South African plants identified and available in GenBank/EBI	Devote coming year to a pro-active and vigorous collecting programme with targeted collectors and joint field expeditions

Output 3 Policy and GMTAs developed and in use	Material transfers running accordingly	MoU including GMTA signed by Kew and NBI on 7 October 2003 and running since then	, ,
Output 4 University-level training	6 people attain PhD or Msc, 40 students receive training	3 students registered for PhD, 4 students for MSc, 26 students received training in biotechnology at NBI	Continue training another 20-40 undergraduates and supervise current PhD/MSc students
Output 5 Publications and seminars	3 scientific papers, 1 manual, press releases	1 scientific paper submitted, 1 field guide published, 3 items published in newsletters, 2 workshops organised	, ,