

Submit by 21 January 2005

## DARWIN INITIATIVE APPLICATION FOR GRANT ROUND 13 COMPETITION:STAGE 2

Please read the Guidance Notes before completing this form. Applications will be considered on the basis of information submitted on this form and you should give a full answer to each question. Please do not cross-refer to information in separate documents except where invited on this form. The space provided indicates the level of detail required. Please do not reduce the font size below 11pt or alter the paragraph spacing. Keep within word limits.

### 1. Name and address of organisation

<b>Name:</b> Royal Botanic Gardens, Kew	<b>Address:</b> Kew, Richmond, Surrey, TW9 3AE, U.K.
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### 2. Project title (not exceeding 10 words)

Cryo-conservation Centre of Excellence for Sub-Saharan Africa (CCESSA)
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### 3. Project dates, duration and total Darwin Initiative Grant requested

<b>Proposed start date:</b> July 05	<b>Duration of project:</b> 3 years				
<b>Darwin funding requested</b>	<b>Total</b>	<b>2005/06</b>	<b>2006/07</b>	<b>2007/08</b>	<b>2008/09</b>
	£168,852	£54,359	£52,859	£61,634	

### 4. Define the purpose of the project in line with the logical framework

Optimisation of appropriate technologies and establishment of CCESSA in KwaZulu-Natal (RSA) to preserve, <i>ex situ</i> , and make available plant species that produce recalcitrant (desiccation-sensitive) seeds. Such seeds cannot be conserved by the conventional methods developed for orthodox (desiccation-tolerant) types. The short-lived nature of recalcitrant seeds means that they are frequently not a component of the soil seed bank, making them far more vulnerable to extinction than those from species that produce orthodox seeds. This is compounded by the destructive utilisation of the parent plants by man, further contributing to the endangered status of such species.
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### 5. Principals in project. Please provide a one page CV for each of these named individuals

Details	Project Leader	Other UK personnel (working more than 50% of their time on project)	Main project partner or co-ordinator in host country
<b>Surname</b>	Dr. Wood		Prof. Berjak
<b>Forename (s)</b>	Christopher Brian		Patricia
<b>Post held</b>	Seed Storage Physiologist (low temperature specialist)		Professor Emeritus
<b>Institution</b>	Royal Botanic Gardens, Kew (Kew)		University of KwaZulu-Natal (UKZN)
<b>Department</b>	Seed Conservation		Biological and Conservation Sciences

### 6. Has your organisation received funding under the Darwin Initiative before? If so, give details

The Royal Botanic Gardens, Kew has received fifteen grants from the Darwin Initiative since 1992.
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**7. IF YOU ANSWERED NO TO QUESTION 6 describe briefly the aims, activities and achievements of your organisation. (Large institutions please note that this should describe your unit or department)**

**Aims (50 words)**

**Activities (50 words)**

**Achievements (50 words)**

**8. Please list the overseas partners that will be involved in their project and explain their roles and responsibilities in the project. Describe the extent of their involvement at all stages, including project development. What steps have been taken to ensure the benefits of the project will continue despite any staff changes in these organisations? Please provide written evidence of partnerships.**

Professors P. Berjak & N.W. Pammenter (UKZN): overseas project co-ordinators involved in programme design and implementation from conceptualisation stage to the establishment of a functional cryo-storage bank; actively engaged in both research and training; at least 50% of their time will be allocated to this project. Dr J.I. Kioko (UKZN): active participant in both the research and training aspects of the programme; 30% of his total time. Dr J. Wesley-Smith (UKZN): expert in cryo-technology of partially hydrated plant tissues; 25% of his total time. Professor D.J. Mycock (University of the Witwatersrand, Johannesburg): particular expertise with vegetative forms of germplasm (e.g. apices, buds and somatic embryos) for cryo-preservation; 20% of his total time. All the named partners will be involved in capacity development. Research Assistants (two); 100% of total time.

A succession plan is in-built, as Dr Kioko is being specifically groomed to continue as the facility manager in the future. Ongoing support from the UKZN is assured, as is that of the International Plant Genetic Resources Institute (IPGRI) and the South African National Research Foundation (NRF) (see supporting documentation).

**9. What other consultation or co-operation will take place or has taken place already with other stakeholders such as local communities? Please include details of any contact with the government not already provided.**

This project currently receives support from UKZN, NRF and IPGRI (which funds selected trainees and students from the rest of Africa). There is on-going collaboration or co-operation with the Tanzanian Tree Seed Programme, the University of Ile Ife, Nigeria, the University of Ghana, Legon and the Institute for Natural Resources in Africa of the United Nations University. The South African National Biodiversity Institute (SANBI) a parastatal institute (department of environment and tourism) actively collaborates with the MSBP in terms of collecting and supplying orthodox seed to the Millennium Seed Bank Project (MSBP) for *ex situ* conservation. The project covered by the current application will complement the work of SANBI as this organisation has neither the expertise nor facilities for cryo-preservation of recalcitrant seed material.

It is intended that the proposed cryo-bank will be able to provide material for re-introduction programmes (the development of 'synseeds' [synthetic seeds, i.e. gel-encapsulated propagatory units such as buds, somatic and embryogenic axes]) as a delivery tool, is pertinent here. As many of the species of interest are heavily utilised in traditional practices, involvement with local communities is inherent in the project.

Positive discussions have been held with the drafters of the South African National Biodiversity Strategy and Action Plan regarding this project.

## **PROJECT DETAILS**

**10. Is this a new initiative or a development of existing work (funded through any source?) Are you aware of any other individuals/organisations carrying out similar work, or of any completed or existing Darwin Initiative projects relevant to your work? If so, please give details explaining**

**similarities and differences and showing how results of your work will be additional to any similar work and what attempts have/will be made to co-operate with and learn lessons from such work for mutual benefits.**

Not a new initiative, it is an extension of the research funded to date by the NRF, UKZN, IPGRI and the MSBP/Kew. However, the aim of CCESSA is to rapidly move from the science of conserving recalcitrant species to its application.

Some work has been done already by UKZN in collaboration with the USDA National Centre for Genetic Resources Preservation. A senior staff member from the Indian National Bureau of Plant Genetic Resources visited UKZN in 2000 to seek advice and guidance to assist in solving problems for a similar facility for Indian species.

**11. How will the project assist the host country in its implementation of the Convention on Biological Diversity? Please make reference to the relevant article(s) of the CBD thematic programmes and/or cross-cutting themes (see Annex C for list and worked example) and rank the relevance of the project to these by indicating percentages. Is any liaison proposed with the CBD national focal point in the host country? Further information about the CBD can be found on the Darwin website or CBD website.**

Target viii of the Global Strategy for Plant Conservation calls for 60% of threatened plant species to be in accessible *ex situ* collections by 2010, and highlights the need for technology development and transfer for species with recalcitrant seeds. Development of this technology, and strengthening local capacity in this field, will support the implementation of articles 5 (5%), 9 (30%), 10 (5%), 12 (20%) of the Convention on Biological Diversity, and the work is specifically directed at the Global Strategy for Plant Conservation theme (40%).

Article 9 of the South African Second National Report to the CBD allocates priority to *ex situ* conservation, and indicates that the resources available for meeting these obligations are 'limiting'. The project covered by this application will contribute significantly to relieving these limitations, particularly through its emphasis on training and capacity building.

**12. How does the work meet a clearly identifiable biodiversity need or priority defined by the host country? Please indicate how this work will fit in with National Biodiversity Strategies or Environmental Action Plans, if applicable.**

The South African National Biodiversity Institute is actively collaborating with the MSBP in terms of biodiversity conservation through (orthodox) seed banking as part of its *ex situ* plant conservation strategy for South Africa. The proposed project will compliment and contribute to this strategy through developing the technology that will permit the cryo-conservation of hitherto un-storable plant genetic resources.

The South African National Biodiversity Strategy and Action plan is still being drafted and is not yet available. However, communications with those responsible for the production of the plan have indicated that more emphasis on *ex situ* conservation of plant genetic resources is important, and that the proposed project will significantly enhance this conservation route. The initial emphasis will be on geophytes (e.g. members of the family Amaryllidaceae) and trees (e.g. *Trichilia emetica*, and the highly-endangered and sought after *Warbutgia salutaris*, among a large number of others) because many of them are threatened or endangered largely because of heavy utilisation in traditional medicine where current harvesting techniques are destructive and unsustainable. The problem is exacerbated by habitat modification and global climate change. Many of the species involved will be from the red-data list.

**13. If relevant, please explain how the work will contribute to sustainable livelihoods in the host country.**

There is tremendous pressure on plants used for traditional medicine (*muthi*), many of which produce recalcitrant seeds which cannot be stored by conventional means. A high proportion of the species involved are over-utilised and highly endangered. Plants will be made available through *in vitro* (micro)-propagation of *ex situ* cryo-preserved material as well as appropriate macro-propagation methods. Such activities should reduce pressure on wild populations and contribute to the alleviation of these problems and hence to the sustainable livelihoods of gatherers, traders and practising herbalists.

As such, the project could have huge potential benefits for the livelihoods of peoples in the host countries (please refer to the *Science* article in the supporting documentation).

**14. What will be the impact of the work, and how will this be achieved? Please include details of how the results of the project will be disseminated and put into effect to achieve this impact.**

The major impact will be the long-term conservation of the genetic resources of plant species producing recalcitrant seeds. This will be achieved through the development of technology to permit successful cryo-storage of germplasm, its distribution and subsequent re-establishment of individual, self-sustaining plants. The work will, therefore, contribute to

re-introduction programmes, conservation of species and provision of sought-after natural resources on a sustainable basis for the sub-Saharan African continent. The results of the project will be disseminated through papers in international peer-reviewed journals, the publication of a cryo-preservation manual and by technology transfer to practitioners from a number of African countries.

**15. How will the work leave a lasting legacy in the host country or region?**

A major 'concrete' legacy will be the centre of excellence, including a fully functional cryo-banking facility. Although this centre will be located in South Africa, and be aimed primarily at serving sub-Saharan Africa, this facility should have a global impact in offering expertise, and in raising the profile of Africa in the biodiversity conservation field. The centre will leave a legacy through capacity building in terms of both graduate scientists and technologists, both in the host country and in the sub-Saharan region generally.

**16. Please give details of a clear exit strategy and state what steps have been taken to identify and address potential problems in achieving impact and legacy.**

At the completion of the project a functional cryo-bank for species producing recalcitrant seeds will be established. Local capacity and the skills of individuals to maintain and extend this facility will be ensured through the cryo-preservation training module and post-graduate capacity building. The association with the Millennium Seed Bank will form a strong base to attract future funding. In addition, the University of KwaZulu-Natal is committed to maintaining and developing CCESSA (see supporting documentation), as evidenced by the level of the matched project funding it is providing. A succession plan is in-built, as Dr Kioko is being specifically groomed to continue and manage the facility in the future.

**17. How will the project be advertised as a Darwin project and in what ways would the Darwin name and logo be used?**

CCESSA is the first project to begin to systematically address the need for a cryo-bank and training facility in sub-Saharan Africa. As such it will be a stimulus and centre of excellence for recalcitrant seed conservation and research work in African extending to many ecosystem types and species of socio-economic and genetic importance. It embodies the spirit of the Darwin Initiative in that it invests in both people and science to conserve bio-diversity currently under threat.

The project will use the name Darwin Initiative and the logo on all general communications, on the web-site, work-shops and the facility itself. Each participating country will develop a media plan to raise awareness of the importance of the facility and fully promote the Darwin Initiative objectives. In addition, peer-review publications will include an acknowledgement of the source of funding and logo (if permitted) for the work reported.

**18. Will the project include training and development? Please indicate who the trainees will be and criteria for selection and that the level and content of training will be. How many will be involved, and from which countries? How will you measure the effectiveness of the training and will those trained then be able to train others? Where appropriate give the length and dates (if known) of any training course. How will trainee outcomes be monitored after the end of the training?**

Training and development constitute a major component of the project. Training at two levels is envisaged: (i) A six week course (equivalent to an Honours module) on cryo-preservation, including theoretical and practical aspects. Whilst concentrating on recalcitrant seeds, students will also be given a basis in cryo-conservation of other forms of plant germplasm. The course will be introduced in 2005, with 4 to 6 RSA students, extended as a training module in 2006 with a similar number from the rest of sub-Saharan Africa and will be subjected to external examination. This course will provide the basis for the production cryo-preservation protocols manual for recalcitrant seeds. The effectiveness of training and technology transfer will be evaluated after the model of Reed *et al.* (2004, *CryoLetters* 25, 341-352). (ii) Post-graduate research training at the Masters and doctoral levels. In the past 4 years UKZN has graduated 4 Doctoral and 5 Masters students in aspects of recalcitrant seed biology (5 theses to be submitted). This number could increase, with growth in student numbers from the rest of sub-Saharan Africa. Research graduates (particularly Doctoral) will be able to train others as part of their capacity development.

**LOGICAL FRAMEWORK**

**19. Please enter the details of your project onto the matrix using the note at Annex B of the Guidance Note. This should not have substantially changed from the Logical Framework submitted with your Stage 1 application. Please highlight any changes.**

Project summary	Measurable Indicators	Means of verification	Important Assumptions
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<p><b>Goal: To draw on expertise relevant to biodiversity from within the United Kingdom to work with local partners in countries rich in biodiversity but poor in resources to achieve</b></p> <ul style="list-style-type: none"> <li>• the conservation of biological diversity,</li> <li>• the sustainable use of its components, and</li> <li>• the fair and equitable sharing of benefits arising out of the utilisation of genetic resources</li> </ul>			
<p><b>Purpose</b> The establishment of a Centre of Excellence for cryo-banking for sub-Saharan Africa.</p> <p>The development and embedding of 'generic technologies' for <i>ex situ</i> collection, storage and utilisation of plant species producing recalcitrant seeds.</p>	<p>Number of requests for research and training placements at CCESSA from African students and from elsewhere.</p> <p>Inward investment (grants) in CCESSA from national and international agencies. Techniques / technologies applied to non-target species by other groups.</p>	<p>Univ. KZN Annual Report; Independent audit reports, e.g. by IPGRI; NRF Annual Report; RBG Kew / MSBP Annual Report. Peer-review papers and other forms of scientific articles / reports.</p>	<p>Institutional support is sustained, resources are not limiting to delivery, and partnerships continue.</p> <p>New protocols are seen as a valuable component of CBD-related conservation action; students / staff apply knowledge routinely on return to their institutes.</p>
<p><b>Outputs</b> Recalcitrant-seeded species in cryo-storage (conserved) and utilisable through propagation and 'extension' activities.</p>	<p>Facility up and running and handling &gt; 15 difficult to store (conventionally) species in 3 years, with 5 species reaching the nursery stage <i>ex vitro</i>.</p>	<p>University KZN records; database entries, and greenhouse and/or field evaluations of performance of plants established from cryo-preserved explants.</p>	<p>Protocols developed are effective and serve as 'exemplars' for other stakeholders. Sufficient material can be made available to sustainable utilisation projects.</p>
<p>Staff and students (particularly from Africa) trained in cryo-biology (both on 6 week honours and post-graduate courses).</p>	<p>Over 3 years, &gt; 10 post-docs and / or graduate students (MSc to Post-doc) given specialised training (6 training weeks per year) and / or research project guidance (continuous, throughout project).</p>	<p>UKZN Science Faculty handbook; Review of successfully completed student theses.</p>	<p>Wide interest by staff / students across Africa for training. Theses available for consultation.</p>
<p>Cryo-preservation technologies refined, through research and made available.</p>	<p>(Y3) Cryo-preservation modules released as hardcopy / electronically, following review of market need; (Y2) 4 publications submitted to ISI-accredited journals.</p>	<p>Review IPGRI list of publications / Kew - MSBP web site; Consult reprints/ preprints of publications submitted and review journal contents pages.</p>	<p>Optimisation of methods is possible.</p> <p>Information as presented meets stringent publication requirements.</p>
<p>Long term <i>ex situ</i> species conservation strategies developed and implemented.</p>	<p>&gt; 45 species collected and evaluated for desiccation tolerance over 3 years; any conventionally bankable species conserved in the Millennium Seed Bank.</p>	<p>Data entered into project data base and, once verified, into the Seed Information Data base on the WWW.</p>	<p>Data standards are to international standard and information is used by appropriate agencies e.g. IUCN, IPGRI.</p>
<p><b>Activities</b> Establishing a functional cryo-preservation centre</p>	<p><b>Activity Milestones (Summary of Project Implementation Timetable)</b> (Y1) Purchase equipment, identify and appoint research assistant staff, write Standard Operating Procedures, design database; (Y3) Germplasm handling for dissemination.</p>		

Delivering research capacity building	(Y1) Design course syllabus; (Y1-3) Complete training needs assessment to identify suitable students, define student projects, mentor and supervise projects, run course and strengthen network of trainees (pan-Africa); (Y3) write cryo manual.
Researching conservation technology	(Y1-3) Develop and assess biotechnological procedures (e.g. explant preparation / culture, optimisation of cryo-protocol, seedling production, monitoring genetic and morphological integrity post cryo-preservation, including via field trials) (Y2-3) Write and submit papers.
Collecting and conserving the target species	(Y1) Species selection; (Y1-3) field studies (phenology of seeding and seed collection), seed processing, storage and post-storage seedling to plant propagation.

**20. Provide a project implementation timetable that shows the key milestones in project activities.**

Project implementation timetable		
Date	Financial year	Key milestones
Jul 05	Apr-Mar	Set up management committee (MSBP, UKZN IPGRI, partners) and initiate database structure.

Jul 05	2005/6	Establish honours cryo-course. Prepare for RSA based planning session. Project leader to review facility and its function. Initiate design of protocols for cryo-storage (methods, data sheets etc.). Start to outline training materials. Identify staff from African biodiversity institutes most appropriate for training (many can be identified from partners of MSBP and UKZN). Finalise 15 species to work on.
Aug 05		Review and purchase equipment. Identify and appoint staff support (2 posts). Write the standard operating procedures and design database. Complete training course assessment and subsequently design course syllabus. Define student projects.
Nov 05		Hold the RSA-based project planning session for the project managers (n=8, see section 23). Establish network participants, prioritise species, match work-plans / activity levels to the needs of the partners. Compile the baseline data for each species and draft reviews. Review existing biotechnological procedures and seek to modify and improve.
Jan 06/07/08		Disseminate schedule and paperwork / protocols agreed at the planning session. Sourcing students and clarification of their associated funding streams.
		Mentor and supervise projects from here-on-in. Start studies (both laboratory based and field work). Registration of MSc students/researchers will occur at this time each year.
		Start research program on 3 species identified; including seed processing, storage and utilisation. Aim for web facility to go live (similar to Darwin DNA bank web-page for South-Africa).
Mar 06	Apr-Mar 2006/7	Deployment of the communications network for researchers that have returned to African country of origin. Complete annual report for DI. Implement handling procedures (from SOP's for germplasm dissemination and utilisation).
Aug 06		Testing and evaluation of potential new germplasm storage and utilisation protocols.
Nov 06		Complete 3 species research and conservation reports.
Jan 07		Assess training of students. Present preliminary work at the International Seed Desiccation Workshop.
Mar 07	Apr-Mar 2007/8	Establish research program on a further 6 species identified; seed processing, storage and utilisation.
Apr 07		Implement 1 <sup>st</sup> phase of the legacy procedures for CCESSA, with some recently qualified students (c. 3 individuals) training/tutoring current students (skills transfer).
Apr 07		Report to committees at 28 <sup>th</sup> congress of the International Seed Testing Association (Brazil).
May 07		Complete annual report for DI and complete 6 species research and conservation reports.
July 07		Planning for cryo-workshop (RSA) with invitations to main international agencies to attend (FAO, IUCN, IPGRI, SANPGRC and African Conservation Foundation) opening of main facility (UKZN) and implementation (e.g. move collections from temporary facility to main, implement restoration protocols). Additional funding to be sought to cover costs of cryo-workshop.
Jul 07		Present major project findings and publicise the Centre of Excellence at Cryo 2007 (Canada).
Aug 07		Prepare for final workshop. Identify speakers from the partners. Consolidate the schedule.
		Distribute the instructions to authors for manuscripts in the proceedings / manual.
Nov 07		Complete annual report for DI and complete 6 species research and conservation reports.
Jan 08		Start research on 6 final species identified.
Mar 08		Assess training of students. Complete research program with the final 6 species identified; including seed processing, storage and utilisation.
Apr 08		Implement 2 <sup>nd</sup> and 3 <sup>rd</sup> phases of the legacy procedures for CCESSA; (i) with recently qualified students (c. 5 individuals) training/tutoring current students (skills transfer) and (ii) Dr Kioko to start to run CCESSA facility.
Apr 08		Submit papers (n=2) for peer-review publication.
Jun 08		Hold final project workshop in RSA, with all 15 species conserved recorded on relevant international documentation and implement the succession plan.
Jun 08		Complete editing of cryo-manual and send to press.
		Implement handling procedures (from SOP's) for germplasm dissemination.
Jul 08		Prepare and submit final report.

**21. Set out the project's measurable outputs using the separate list of output measures.**

PROJECT OUTPUTS		
Year/Month	Standard output number (see standard output list)	Description (include numbers of people involved, publications produced, days/weeks etc.)
Jul 05	7, 12A, 17A	Initiation of CCESSA management committee and workshop

Aug 05	4C, 4D, 8	/ database structure, one person week, 8 people. Planning workshop in RSA (8 people) for 2 weeks, 16 person weeks.
Aug 05	11B	Submit review on baseline species.
Aug 05	15A, 15B, (18A, 19A if possible)	Press release in participant countries announcing start of project (c. 5 releases).
Aug 05	15C (18B, 19B if possible)	National and International press release in Kew Scientist and Samara.
Jan 06	1A, 2, 3	MSc, PhD and post-doc projects started(5-6 students, 2 to 3 years).
Nov 06	10	3 species reports submitted.
Jan 07	14B	Present project at the International Seed Desiccation Workshop (RSA).
Jan 07	1A, 2, 3	Graduation of previous students and new MSc, PhD and post-doc projects started (5-6 students, 2 to 3 years).
Apr 07	21	1 <sup>st</sup> legacy phase of CCESSA initiated, including facility, 1 facility leader and numerous support activities.
Apr 07	14B	Present CCESSA at 28 <sup>th</sup> congress of the International Seed Testing Association (Brazil).
May 07	10	Research reports submitted on 6 additional species.
Jul 07	14B	Present major findings and publicise at Cryo 2007 (Canada).
Jan 08	1A, 2, 3	Graduation of previous students and new MSc, PhD and post-doc projects started (5-6 students, 2 to 3 years).
Apr 08	11B, 17B	Submit at least 2 papers for peer-review publication, complete database and web facilities.
Jun 08	10	Research reports submitted on final 6 species.
Jun 08	6A, 7	Implement future germplasm dissemination procedures (involving c. 5 to 10 graduates from pervious training courses, partner country based).
Jun 08	10, 11A, 11B	Complete cryo-manual and send to press.
Jul 08	13A, 13B, 14A, 14B, 15A, 15B, 15C, 21	Conserve bankable species in CCESSA facility as long-term investment (up to 15 species) with associated press releases.
	2, 3	Prepare and submit final reports. Graduation of current students after project completion.

## MONITORING AND EVALUATION

**22. Describe, referring to the Indicators in the Logical Framework, how the progress of the project will be monitored and evaluated, including towards delivery of its outputs and in terms of achieving its overall purpose. This should be during the lifetime of the project and at its conclusion. Please include information on how host country partners will be included in the monitoring and evaluation.**

Four 6-monthly reports of the partners (Sept. 2005; March 06; Sept 06; March 07; Sept 07; final technical report July 08) AND 3 yearly reports and a final project report from the co-ordination team (April 06; 07; 08 and July 08) will be produced. Their timeliness will be noted. One proceedings volume for the final workshop (June 08). Three evaluations (questionnaires) of the training benefits, one each in 2006; 07; 08 for c. 5 - 6 students (per course in RSA and participating countries). Appropriate application of techniques will be judged by consulting / monitoring reports throughout. Data quality in reports will be reviewed regularly by experienced members of the Management Team and ultimately assessed by journal referees on submission of manuscripts. Constant face-to-face contact with all partners over the three years will not be possible and thus e-mail / fax exchange will be integral to the monitoring process regarding technical issues and more general matters. Dissemination of results and networking will be monitored by: 1) careful examination of species reports (up to 15) written for those preserved; 2) noting the number of papers submitted for publication (at least 2); 3) internet web site establishment and use.