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Crop wild relative

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Conserving plant genetic resources

for use now and in the future

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Crop wild relative

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Editorial

Crop wild relative Issue 3 is brought to you in PGR Forum's third and final year, but with so much to report, two more issues are planned before the end of the year, and the good news is that due to the popularity of the newsletter, funding has already been secured for a further three issues after the end of the project. It is also anticipated that *Crop wild relative* will continue to be published under the auspices of the Crop Wild Relative Specialist Group (CWRSG) of the IUCN Species Survival Commission, which will be inaugurated this year.

It is now less than six months until the First International Conference on Crop Wild Relative Conservation and Use (see page 18), which will be a landmark in PGR conservation, highlighting crop wild relatives (CWR) as a critical but neglected resource. A varied and stimulating programme is under preparation, including topics not covered by PGR Forum such as *ex situ* conservation and use of CWR. Combined with the backdrop of the ancient Valley of Temples, the azure of the Mediterranean Sea and the unrelenting hospitality of the Sicilian people, the Conference is sure to be a huge success. Whether you are a conservation manager, plant breeder, policy-maker, information manager, researcher or other individual interested in crop wild relatives, be sure not to miss this historic event. For information and registration details visit http://www.pgrforum.org/conference.htm

The activities of PGR Forum have centred around a number of workshops, each one addressing specific issues related to CWR conservation. Four three-day workshops have taken place to date, the latest being "Genetic erosion and pollution assessment methodologies", held on Terceira Island, the Azores, Portugal, 8-11 September 2004 (see page 13). The final workshop in the series, "Threat and conservation assessment" will take place in Korsør, Denmark, 27-30 April 2005, and will be reported on in the next issue of *Crop wild relative*.

It is through these intensive and productive workshops that PGR Forum has been able to achieve so much in a relatively short time. A number of publications are under preparation as a result of the workshops and associated research, including a manual in the IPGRI Technical Bulletin series that addresses practical guidelines for *in situ* CWR population management and monitoring, and a number of articles to be published in peer-reviewed journals such as *Biodiversity and Conservation*. In addition, the proceedings of the First International Conference on Crop Wild relative Conservation and Use will be published by CAB International (http://www.cabi.org/), a copy of which will be included with Conference registration. It is anticipated that these publications will have a significant impact on CWR conservation and exploitation, both within and outside Europe.

While we are waiting for these major peer-reviewed publications to appear on our shelves, *Crop wild relative* continues to provide a more immediate medium through which to publish articles and news items addressing CWR conservation and use. Issue 3 provides us with an insight into a wide range of CWR topics, including an article raising awareness about wild plants being assessed for their use in sewerage treatment (see page 19). We also learn about the threatened CWR *Apium repens* in Hungary (page 11), the importance of monitoring evolutionary change in forest tree species (page 4), how changes in grassland management practices are altering the landscape and its component plant species in Norway (page 15), the relationship between ecogeographic and genetic variation in populations of wild *Brassica* in England and Wales (page 20), and a GEF-funded project addressing the conservation of CWR in five countries: Armenia, Bolivia, Madagascar, Sri Lanka and Uzbekistan (page 7).

We hope you enjoy reading this issue and encourage you to distribute the newsletter amongst your colleagues. Issue 4 will follow closely behind Issue 3, and will focus on delivering a draft programme and cultural event details of the First International Conference on Crop Wild Relative Conservation and Use.

Please continue to send us your articles and items for future issues of *Crop wild relative*!

Tünde Kovács

Above: Creeping marshwort (*Apium repens*), Bokod, Hungary. This strictly protected species is a very rare wild relative of cultivated celery (*Apium graveolens*) present in the Hungarian flora (see article, page 11)

Monitoring evolutionary changes in forest trees: general concepts and a case study in European black poplar (*Populus nigra* L.)

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Conservation of genetic resources: the need for a global and dynamic approach

Forest genetic resources exist in a continuous gradient of population types from cultivated to wild, which interact through gene flow or exchange of pathogens (Lefèvre, 2004). The intensity of domestication, which can be defined as the genetic changes of anthropogenic origin in the cultivated pool, also varies from one species to another. In Europe, poplars (*Populus* L.) (Fig. 1) are an example of a highly domesticated resource; most cultivated populations exist in monoclonal stands of interspecific hybrids, while the wild riparian populations go extinct due to habitat deterioration. For species like spruce (*Picea* spp.) or pines (*Pinus* spp.), domestication reaches the point of improved population varieties, whereas for other species, like oaks (*Quercus* spp.) and beech (*Fagus* spp.) for example, domestication is generally restricted to seed transfer from selected "natural" stands. Finally, there are some species like mountain ash (*Sorbus* spp.) or pear (*Pyrus* spp.), generally with a scattered distribution, that are only exploited as a wild resource with no direct genetic improvement. The European forests consist of a mixture of cultivated and wild genetic resources that must be considered collectively. Preserving forest genetic resources in the long run relies on three complementary activities: (i) sustainable management of the forest, (ii) protection of sites and reserve areas, (iii) specific programmes for the conservation of target genetic resources.

The term "conservation", when applied to genetic resources, can be misleading. Genetic resources are subject to constant change (e.g. genetic erosion and genetic pollution) and the current state of *in situ* diversity cannot therefore be considered as an objective to be maintained *per se*. Current population size, genetic diversity or even adaptedness of the resource are not stable nor ideal parameters, but rather instantaneous values resulting from dynamic processes. Therefore, the question is how to monitor the dynamics of the whole system. This dynamic perspective of adaptation is relevant to all organisms, but particularly in the case of forest trees when the time needed to reach population equilibrium (i.e. hundreds of generations or thousands of years) exceeds the time scale of environmental stability.

> "Preserving forest genetic resources in the long run relies on three complementary activities: (i) sustainable management of the forest, (ii) protection of sites and reserve areas, (iii) specific programmes for the conservation of target genetic resources"

General concepts regarding genetic erosion and hybridisation-introgression

Monitoring evolutionary changes requires a measure of the rate of evolution. The concept of effective population size gives such a measure. The term is sometimes confusing; see Nunney (2000) for a detailed discussion on its significance. It comes from Wright-Fisher's model population, submitted to genetic drift as a single evolutionary force that erodes genetic diversity and increases inbreeding, with no mutation and no selection. Under

Figure 1. European black poplar, *Populus nigra* L., an example of a CWR in forestry with a conservation plan at the European level

a series of assumptions (hermaphrodism, panmixia etc.), the rate of evolution in this model population, regarding either diversity or inbreeding, is entirely determined by its effective size N_e. This theoretical population is the reference to which any real population can be compared: the effective size N_e of an actual population is the size of the model population that would have exactly the same rate of evolution. In other words, N_e is a unit for measuring the decrease in diversity or the increase of inbreeding. More precisely, the evolutionary changes are proportional to $1/N_e$: no evolution at all (purely theoretical) would be an infinite effective size; a constant rate of evolution corresponds to a constant N_e; whereas an acceleration of genetic erosion corresponds to a decreasing N_e. (Fig. 2)

To understand the value of the parameter N_e, we can start from Wright-Fisher's model population and eliminate its assumptions one at a time. It can be shown how N_e decreases, i.e. evolution accelerates, under several factors: biological factors (true or partial dioecism, departure from panmixia), demographic factors (variation of population size across generations, variance in mating success, generation overlap) and environmental factors (selection). Human activities interfere with each of these biological, demographic and environmental factors; the question then is how much N_e is affected and how much the evolutionary changes are re-oriented. In practice, the objective is not to estimate N_e for a real population, but rather to use it to compare different strategies or practices. For instance, a silviculture that will enhance the variance in mating success will decrease N_e and therefore increase inbreeding.

Concerning the concepts of hybridisation and introgression, a complete review can be found in Allendorf *et al.* (2001). In trees, as for most plant genetic resources, the concepts must be considered in a broad sense, i.e. including the intraspecific level: typically, gene flow between wild and cultivated populations. Here, we are more interested in the anthropogenic impact on gene exchanges between populations. Due to their long generation time, and the relatively recent human impact, trees will rarely reach the point of introgression, which requires several generations of backcrossing. The central question is the impact of gene flow on the fitness or the adaptedness of the population: a comprehensive synthesis on this issue was made by Lenormand (2002).

The impact of gene flow results from balanced effects (Fig. 3). On the positive side of the balance, say "reinforcement" in reference to the practice sometimes used in conservation biology, introduction of an alien pool can increase the actual population size (demographic rescue for a local population in sharp decline), and it can also increase genetic diversity and reduce inbreeding. By contrast, on the negative side, say "extinction", it can lead to demographic swamping (the new generation in entirely made of hybrid forms that will reproduce badly themselves), it increases migration load (bring in maladapted genes), and it can reduce the actual population size (if the alien or the hybrid becomes super-fit). The outcome of balanced effects will vary from one situation to another. However, some general tendencies can be hypothesized (Lenormand, 2002). The negative impacts of the "extinction" side of the balance are reduced when: (i) the local taxon is not rare, (ii) reproductive barriers are strong, (iii) generation time is long, (iv) selfing or vegetative propagation occurs, (v) differential selection is enhanced.

Specific features for trees

Genetic markers reveal higher genetic diversity for trees than any other organism, including both global diversity at the taxonomic level and within-population diversity (Hamrick, Godt and Sherman-Broyles, 1992). Moreover, the genetic differentiation among tree populations is weak. Considering that post-glacial re-colonisation of trees was rapidly achieved in few generations, the observed pattern of genetic diversity is explained by intensive gene flow among populations. The importance of migration as an evolutionary force in trees is further enhanced by the long juvenile phase: when a new site is colonised, migrants accumulate over years before they start to reproduce themselves, thus reducing the founder effect (Austerlitz *et al.*, 2000).

In spite of recent colonisation and important gene flow, tree populations rapidly develop local adaptation, and clinal variation is often observed for adaptive traits, e.g. phenology in oaks

"In practice, the objective is not to estimate N_e for a real population, but rather to use it to compare different strategies or practices. For instance, a silviculture that will enhance the variance in mating success will decrease N_e and therefore increase inbreeding"

(Ducousso, Guyon and Kremer, 1996). The rapid effect of selection is also observed on recently transplanted tree populations (Skrøppa and Kohman, 1997). Local adaptation is a dynamic process where natural selection is balanced by other processes that prevent the population from reaching an "optimal" genetic composition: gene flow (migration load), interaction among species and temporal fluctuations of environment. Therefore, populations maintain a high level of genetic diversity for adaptive traits, which is essential for future evolution. Climate change will directly affect the current generation of trees and their progenies. In response to this environmental change, three adaptive strategies are expected for forests: individual phenotypic plasticity is an immediate response, then, rapid genetic evolution can occur in few generations, and, finally, migration of populations will probably take more time than the other processes. A comprehensive study of adaptive potential in *Pinus contorta* populations was published by Rehfeldt, Wykoff and Ying (2001).

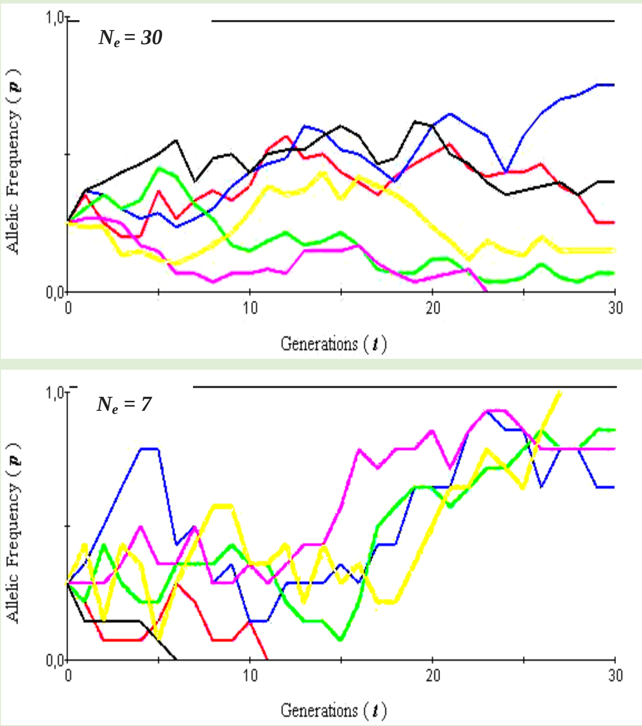

Figure 2. Genetic erosion: the effective population size N_e is a standardised parameter measuring the rate of evolution of the genetic diversity in a population, i.e. erosion of diversity (top) or increase of consanguinity. The lower the value of N_e, the more intense evolution of the diversity. Infinite N_e means no evolution, constant N_e means a constant rate of evolution, decrease in N_e means an acceleration of genetic erosion. Rather than monitoring the genetic diversity, we can monitor the evolution of diversity by predicting what human activities will increase or decrease N_e.

Genetic erosion, the reduction of within-population diversity, can directly affect the adaptive potential of our forests and should be avoided. Based on recent studies of the impact of domestication in crop plants, we can conclude that breeding and selection activities generally do not represent a major threat of genetic erosion in trees, except in the particular cases of low initial diversity (Lefèvre, 2004). Population management, not only restricted to silviculture, has a direct impact on demographic parameters: life cycle, dispersal, mating system, survival and mating success. It should not affect the processes that maintain a high level of diversity within tree populations. This is a rather general statement, but all population management practices have their own advantages and drawbacks, and each case must be considered individually (see Lefèvre (2004) for a review). Genetic erosion is itself a dynamic process that can be accelerated or slowed down by population management (Lefèvre *et al*., 2004).

Wolf, Takebayashi and Rieseberg (2001) proposed a general frame for monitoring the risk related to genetic pollution. It must be stressed that introductions, landscape fragmentation and habitat disturbance influence the process of hybridisation and introgression. The first step is risk assessment. Assessing the occurrence and frequency of hybridisation often raises technical difficulties, particularly in the case of intra-specific hybridisation. Risk assessment is also needed to estimate the variation of frequency among cohorts, as well as the relative fertility of hybrid types. The second step is to reduce the impact of hybridisation if considered as a risk. This can be achieved in several ways: through elimination of hybrids and invasive species, or by improving habitat in order to enhance competition and differential selection between local and hybrid forms. In particular, the impact of commercial seed transfer on local genetic resources depends both on the origin of the seeds and the destination site (Lefèvre, 2004); in an optimal situation, the risk of maladaptedness should be avoided through sufficient preliminary tests; a higher risk is expected when the local resource has small N_e.

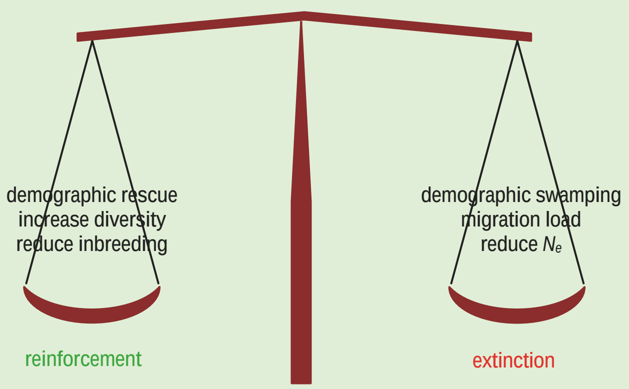

Figure 3. Gene flow, which results from natural migration or artificial transplantation followed by hybridisation, has a many-fold impact on the local genetic resources. Depending on the size of local populations, on the genetic diversity of the migrant pool and the adaptedness of the migrant pool to local conditions, the balanced effects will result in positive or negative impact. The risk of extinction of the local germplasm is reduced when the local taxon is not rare, when reproductive barriers are stong, when generation time is long, when selfing or vegetative propagation occurs or when differential selection is enhanced.

> "large naturally regenerating and self-sustainable poplar stands are rare in Europe, but small stands can also play a role, and, in other cases, ecological management can promote natural regeneration"

Populus nigra as a case study

The European black poplar, *Populus nigra* L., is a genetic resource of interest for breeding. Natural populations have severely declined over Europe due to habitat disturbance of riparian areas. A network of the EUFORGEN programme was specifically dedicated to this resource (see http://www.ipgri.cgiar.org/networks/euforgen/Networks/Poplars/PN_home.asp). Complementary approaches for its conservation can be applied: *ex situ* collections for the areas where natural populations have disappeared or where natural regeneration does not occur anymore, *in situ* management of conservation stands whenever possible, or reserve areas of riparian forests. Three objectives are generally assigned to *in situ* conservation of forest genetic resources: (i) ensure the regeneration in sufficient quantity, (ii) ensure the genetic quality of the regeneration for further evolutionary processes, (iii) preserve the ecological and genetic characteristics. The EUFORGEN Network recently published guidelines for *in situ* management of this pioneer species that are briefly introduced hereafter (Lefèvre *et al*., 2001).

The first point in these guidelines was to identify the different population types that can be found today in Europe, and assign to each of them a potential specific role in the conservation plan. Indeed, large naturally regenerating and self-sustainable poplar stands are rare in Europe, but small stands can also play a role, and, in other cases, ecological management can promote natural regeneration. The second point was to inform on the interaction between ecosystem dynamics and poplar population biology. We can expect that for most pioneer species, population dynamics will mainly be controlled through the dynamics of the ecosystem rather than through direct control of demography. For instance, regeneration will generally not take place where the reproductive individuals occur, but in recently opened areas. The third point was to make some recommendations for restoration projects, as already planned in some countries. Finally, we addressed the question of criteria and indicators to be used for monitoring *in situ* conservation units. Related to these guidelines, a joint European Research project was launched that investigated the following topics of interest for conservation, among other research tasks: gene flow, mating system, clonal propagation, introgression (Van Dam and Bordacs, 2002).

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A global initiative to conserve crop wild relatives *in situ*

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"*In situ* conservation of crop wild relatives through enhanced information management and field application" is a UNEP/GEF supported project that addresses national and global needs to improve global food security through effective conservation and use of crop wild relatives. This multi-faceted five year project was launched in 2004 and brings together five countries and six international organizations to manage and make use of the wild relatives of vitally important crops.

The natural populations of many crop wild relatives are increasingly at risk and they are at present poorly conserved, for a range of reasons. There are technical problems involved in developing conservation plans for such a diverse range of species with different biological characteristics, ecological requirements, conservation status and uses. There are also political, administrative and infrastructural problems that limit effective *in situ* conservation actions. In many cases, collaboration between different ministries, agencies or institutions is required where there is no tradition of collaboration and, in fact may even be a history of competition. While many countries already have conservation initiatives in place (e.g. gene banks and protected areas) few of these target crop wild relatives (Meilleur and Hodgkin, 2004). An assessment of *in situ* conservation of *Lupinus* spp. in Spain, for example, showed that protected areas do not consider crop wild relative populations unless they are an endangered species (Parra-Quijano, Draper and Iriondo, 2003). Undoubtedly, however, a major limitation is in the capacity to bring together and use information that does exist. A number of studies have shown that substantial amounts of information often exist (e.g. Thormann *et al*., 1999) but that it is dispersed among different institutions and agencies in different countries and international organizations, and is often biased towards food crops

Annie Lane

Above: Wild vanilla *Vanilla* sp., Ankarafantsika National Park, Madagascar; an important spice common in markets and on the street

The project was developed to address national and global needs to improve conservation of crop wild relatives, focusing on improved management and use of information on these species. It brings together five countries - Armenia, Bolivia, Madagascar, Sri Lanka and Uzbekistan - with IPGRI as the project manager, and five other international conservation agencies - the Food and Agriculture Organization of the United Nations (FAO), Botanic Gardens Conservation International (BGCI), the United Nations Environment Programme's World Conservation Monitoring Centre (UNEP-WCMC), IUCN - The World Conservation Union and the German Centre for Documentation and Information in Agriculture (ZADI) - to enhance the conservation status of selected crop wild relatives in each country. Each of the countries has significant numbers of important and threatened taxa of crop wild relatives. Each is also among the world's biodiversity hotspots; places that have the highest concentrations of unique biodiversity on the planet. They are also the places at greatest risk of loss of diversity.

Project development and implementation

The preceding two year design and development phase of the project analysed the conservation situation for crop wild relatives in the five countries. It was found that relatively little is known of the conservation status of these species, no management plans have been developed for reserves with such species in mind, no modern information management systems exist, and no *in situ* conservation projects or monitoring activities targeted to crop wild relatives were currently in place. All of the partner countries expressed their desire to improve the conservation and wise use of these important resources in a sustainable and cost-effective way. To achieve this, they decided to use approaches that maximize the use of existing information and conservation resources in ways that are widely applicable to the different taxa that occur within their borders.

This first year of project implementation is dominated by activities to establish infrastructure, processes and personnel. The first International Steering Committee (ISC) meeting was held in Sri Lanka in July 2004 to refine budgets and work plans and to confirm the course for the first year. Each country has now established a project management unit to coordinate in-country activities and work with the global project management unit established at IPGRI. An Information Management Committee (IMC) has been formed to drive the information components of the project and its first workshop and meeting were held in October 2004. Representatives from each of the five countries and six international organizations attended as well as a representative from PGR Forum. A Technical Advisory Committee will soon be established to provide advice and guidance to the ISC on technical matters.

Other activities during the first year will include the development of collaborative agreements for exchange of information both within and between countries and for the coordination of *in situ* conservation activities within countries. Information will be collected on the current level of awareness of crop wild relatives and the conservation and management status of wild relative populations in protected areas so as to provide a baseline

Annie Lane

Above: Wild pepper (*Piper* sp.) in the Limestone grottos of Walvulpana, SE of Colombo, Sri Lanka

against which to measure project performance and impacts of activities in relation to these aspects. In this regard, a detailed monitoring and evaluation plan will be developed collaboratively by the national and global project management units.

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Key elements

The project has four major components, the first two of which focus on the systematic compilation, access and use of information related to crop wild relatives. Application of this information will significantly enhance the development of effective *in situ* conservation and monitoring strategies for crop wild relatives, which is the major focus of the third component, and in raising awareness, the fourth component.

<u>Component 1. International information management system.</u> An information portal dedicated to crop wild relatives will be developed to serve as a gateway for access for the global community allowing users to search for information through a single web address. CD-ROM products will also be distributed to users throughout the world who may not have access to the internet. The system will bring together information from available national and international sources on the identity, status, distribution and potential use of crop wild relatives. The five participating countries will provide information from their systems as the project develops and it is hoped that other countries will also provide their information in due course. The national partners will also test the effectiveness of access and use of the international system to support country conservation decisions.

The major activities under this component are:

(a) Design of the system will involve all international partners and national information experts. The design of the International Information System is co-funded by GTZ, an international cooperation enterprise for sustainable development. The design process will also address issues of ownership, custodianship, access, use, exploitation and intellectual property.

(b) Joint terminology which will provide definition of relevant terms, their specification and the relationship between them. The main entry point for accessing information is likely to be species name. Sixty four crop genera comprising about 12, 000 species have at this stage been identified as the focus of the project, though this number is likely to increase with inclusion of a greater number of crops listed in Annex 1 of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), which came into force on June 29, 2004,

Above: Participants at the First Information Management Workshop of the GEF-funded project, *In situ* conservation of crop wild relatives through enhanced information management and field application, held at IPGRI Headquarters, Rome, 4-7 October 2004

for which multilateral exchange agreements are envisaged. The terminology development work will directly support national information management in the five partner countries.
(c) Development of infrastructure that will provide a cost-effective internet based system that can host information gathered and compiled by partners. Analytical tools suitable for spatial information and geographic analysis will allow the distribution of crop wild relatives to be visualized and related to variables such as climate, edaphic factors and land use and social-economic factors such as human population and poverty.
(d) Content development will ensure that information maintained by relevant international and national organizations is made available to users. Resources for content development will be primarily targeted to national partners to support their information access work.

At the first IMC meeting it was agreed that the system design would be based on the model used by Global Biodiversity Information Facility (GBIF). Information categories were agreed and work was initiated on the development of descriptors. It is expected that the work on descriptors will be finalized by the end of 2004 and countries and international organizations can then begin to enter data and modify information categories and their descriptors.

<u>Component 2. National information systems.</u> In all the five partner countries information exists in herbaria and *ex situ* gene banks that can be used to determine the likely location of populations of species of crop wild relatives. Information on the extent and distribution of protected areas is also available from natural resource management agencies, and information on the use of crop wild relatives can be found in institutions attached to the Ministry of Agriculture, universities and colleges. The country partners will be actively involved in the development of the international system and will ensure that procedures can be used by national institutes and organizations. Information from national sources in the five countries will be brought together using common protocols and procedures for information sharing and data management to ensure effective movement of information between international and national systems, as well as between national agencies.

The national partners will analyze existing information holdings, establish necessary infrastructure, develop appropriate hardware and software systems and national data exchange protocols and ensure that the information is available to the information system. Their systems will include aspects of species biology, ecology, conservation status, distribution, crop production potential, local community uses and existing conservation actions and information sources on crop wild relatives.

<u>Component 3. Enhanced capacity and conservation actions.</u> Lack of capacity, including the absence of an effective operational framework and national plans to deal specifically with conservation of crop wild relatives, has been identified as a significant obstacle to their conservation and use. This component contains a range of activities to improve country capacity to effectively conserve and use crop wild relatives. A solid legal structure is needed and decision-making procedures for identification of priority conservation actions need to provide for the participation of all stakeholders. The legal framework as it relates to *in situ* conservation of these species will be reviewed in each country. Recommendations will be made where new or modified legislation is required. Similarly, benefit-sharing practices are to be framed into legal rules that set out entitlements. Supporting the development of an operational framework will be a series of training activities and these are will include information management, Red Listing procedures, participatory approaches and benefit sharing issues.

The partners in each country will implement and monitor conservation strategies that are needed to conserve priority crop wild relatives *in situ*. Countries will undertake ecogeographic surveys and analysis on three to five taxa and use this information to refine procedures for using spatial information as a tool in conservation management and monitoring. Specific conservation actions will be identified by integrating information on the species themselves, information on

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"Countries will undertake ecogeographic surveys and analysis on three to five taxa and use this information to refine procedures for using spatial information as a tool in conservation management and monitoring"

the existing conservation actions and the use of these species at local and government level. A selected set of actions that are identified as high priority will be implemented and tested for operational effectiveness and sustainability. An action plan will be developed for at least one protected area per country that contains crop wild relatives, and at least two significant *in situ* crop wild relative conservation demonstration projects will be implemented and assessed with a view to their application as national (and potentially international) models for sustained conservation. Priority will be given to working with wild relatives of crops of importance to the partner countries. However, wild relatives important for crop improvement in one country may only occur in other countries. This international dimension will be reflected by working on wild relatives of a common agreed list of crops. In this way, the outcomes will provide globally relevant solutions to improving conservation of crop wild relatives.

Component 4. Public awareness. Awareness concerning the need for conservation of plant genetic resources (PGR) and especially crop wild relatives is relatively recent. Awareness of PGR has increased since the approval of the ITPGRFA but knowledge of the value of crop wild relatives to plant diversity and sustainable livelihoods is low. The output will raise awareness within the countries and internationally of the importance of considering crop wild relatives and their value for improving agricultural production. Country and international partners will work together to develop international public awareness activities that ensure that project outputs are made available to conservation workers in non-target countries. These activities will be targeted at various sectors involved in PGR such as policy makers, conservation managers, plant breeders and local users.

The outcomes of the project will be widely disseminated nationally and globally and successful strategies (best practices) will be readily transferable to other countries with significant populations of crop wild relatives. In this way, global efforts to conserve biological diversity in general, and crop wild relatives in particular, will be accelerated and optimized for the benefit of both the global community and local users.

It is clear that the initiatives developed under this project have much in common with the aims and objectives of PGR Forum. Thus, there is great potential to share information, experiences and lessons and to generate synergistic benefits to both projects and to similar initiatives around the world. We expect that the UNEP-GEF Crop Wild Relatives Project will provide a sustainable information and decision-making framework for current and subsequent work on the conservation of crop wild relatives and in doing so make a significant global contribution to their conservation and use. Whilst wild relatives have already contributed many useful genes to crop plants, there is great potential to significantly improve our knowledge, information dissemination systems and, ultimately, effective use of these species. This will lead to further improvements in crop production and, critically for subsistence farmers, reduced risk of crop failure and improved food security.

Shelagh Kell

Above: Participants at the First Information Management Workshop of the GEF-funded project, *In situ* conservation of crop wild relatives through enhanced information management and field application, held at IPGRI Headquarters, Rome, 4-7 October 2004

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Distribution of creeping marshwort (*Apium repens* (Jacq.) Lag.) populations in Hungary

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Apium repens (Jacq.) Lag. is a small creeping perennial umbellifer, forming rosettes of simply-pinnate leaves from which runners or stolons extend (30 cm) with roots at the nodes. It grows in open, wet grasslands subject to winter flooding, typically along rivers, and flowers from July to August. It occurs through central and southern Europe, and North Africa. Creeping marshwort is an endangered species in Europe, growing in small populations in France, Belgium (Ronse and Vanhencke, 2004), The Netherlands, Germany, Switzerland, Poland, Hungary, Spain, United Kingdom, Italy and the Czech Republic. Recently, on the left bank of the river Danube (Slovakia) the species appeared not far from the village of Dobrohošt (Ohrádková, 1998). It is also found in the Canary Islands and in Morocco, where it grows along watercourses in the Atlas Mountains. It is considered as an endangered species throughout its distribution area (Vogel and Büschner, 1988) and mentioned in the Bern Convention annex II, the EU Habitats Directive annex IV (Directive 92/43/EEC), and CORINE Biotopes Programme. In Hungary, this species is protected by Law (Act No. LIII. of 1996 on Nature Conservation). *Apium repens* closely resembles certain varieties of the related fool's watercress, *A. nodiflorum* (L.) Lag., leading to confusion over the true status of *A. repens*. Recent taxonomic investigations appear to indicate that these species coexist, but that they do not hybridise, although the two species may be almost indistinguishable in the field. However, doubt has been raised on the reliability of the conclusions drawn from genetic tests (Grassly, Harris and Cronk,1996). *A. repens* is sometimes also confused with *A. inundatum* (L.) Rchb.f., especially with its form *isophyllum* (Sonder) Thell. (Reduron and Wattez, 1986).

Distribution in Hungary

The strictly protected creeping marshwort (*Apium repens*) is a very rare wild relative of cultivated celery (*Apium graveolens* L.) present in the Hungarian flora. This diploid (2n=22) wild species only occurs in certain wet habitats. In the Carpathian basin, it has so far been reported from a few locations along and near the Danube valley (Szigetköz, Csepel, Danube-channel) and from sites near the river Drava (Table 1). The first report on the occurrence of creeping marshwort in Hungary (Akasztó) was published by Menyhárth in 1877. Among the herbarium specimens inspected the latest specimens were collected by Á. Boros near Sükösd and by J. Papp from Soroksár in 1961. From recent studies, this species is known to also occur at Dunaszeg (Molnár and Pfeiffer, 1999), Sükösd (Molnár and Pfeiffer, 1999), Császártöltés (Kun *et al*., 1999), Paks (Voigt, 2000, Farkas and Molnár, 2001), Bogyiszló (Farkas, 2000) and Bokod (Riezing, 2001), where population sizes have shown wide fluctuation from year to year (Fig.1). In Szigetköz region (Lesser Plain), f. *repens terrestris* form of this taxon has been found on sandy-pebbly soil at the southern bank of the lake Dunaszeg (Molnár and Pfeiffer, 1999). This species was earlier reported from this location by Polgár (1941). During recent floristic research in the northern part of the Vértes mountains, *A. repens* was discovered near the village of Bokod by Riezing (2001). Along the Danube in the south-west part of Duna-Tisza midregion, it is grown near Császártöltés and Sükösd villages. Since 2003, the Institute for Agrobotany has started to revisit some habitats (Bokod, Paks) of this rare species in order to identify populations suitable for *in*

Figure 1. Occurrence of *Apium repens* in Hungary

Tünde Kovács

Figure 2. The natural habitat of creeping marshwort (*Apium repens*) in a former river bed of the Által-ér near Bokod, Hungary

> "The strictly protected creeping marshwort (*Apium repens*) is a very rare wild relative of cultivated celery (*Apium graveolens*) present in the Hungarian flora"

Tünde Kovács

Figure 3. The tiny, white flowers of creeping marshwort (*Apium repens)* near Paks, Hungary

situ conservation. In 2003, a few dispersed populations were located in a former riverbed of the Által-ér near the village Bokod (Fig. 2). Near Paks this species occurs in Ürge-mező marshland (Fig. 3), periodically grazed by sheep.

Protection status

Creeping marshwort is protected by law in Hungary. Some of the known sites also occur within protected or landscape conservation areas. The Dunaszeg site belongs to the Szigetköz landscape conservation area. The Paks locations of creeping marshwort populations including Ürge-mező grassland, are within the Dél-Mezőföld landscape conservation area. The nature conservation area around Császártöltés is devoted to the preservation of the remains of the sandy and loess plains natural habitats and wetlands, including sites of creeping marshwort. The Császártöltés and Sükösd locations in Bács-Kiskun county are supervised by the Kiskunság National Park. For *in situ* maintenance of this rare species, it is necessary to apply the following measures outside and within landscape conservation areas:

1. Research and *ex situ* conservation:
   - Assessment of current occurrence of the species
   - Vegetative propagation and *ex situ* conservation
2. *In situ* conservation in collaboration with national parks administration:
   - Protection of habitats
   - Maintenance of populations and existing genetic variation

Conclusion

The maintenance of natural habitats of *Apium repens* is difficult as they are situated on periodically flooded grasslands with uneven groundwater levels, and some of these locations are grazed at different intensities. Further detailed studies are necessary to reveal the influences of these factors on the survival and genetic composition of creeping marshwort populations in natural habitats. Measures should be taken to assess the existing genetic variation and arrange for back-up conservation in *ex situ* collections.

Acknowledgements

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Site	Collecting date	Collector	Lat.	Long.	Alt. asl
Akasztó	1877.	Menyhárth L.	46° 41'	19° 12'	90
Dunaszeg	1908.09.11	Polgár S.	47° 46'	17° 33'	114
Soroksár-Dunaharaszti	1915.06.17	Degen Á.	47° 23'	19° 07'	96
Soroksár-Dunaharaszti	1915.08.07	Degen Á.	47° 23'	19° 07'	96
Soroksár-Dunaharaszti	1915.08.26	Trautmann R.	47° 23'	19° 07'	96
Dunaszeg	1919.08.24	Polgár S.	47° 46'	17° 33'	114
Dunaszeg	1920.07.	Polgár S.	47° 46'	17° 33'	114
Sükösd	1921.06.10	Greinich F.	46° 16'	19° 00'	96
Soroksár-Dunaharaszti	1921.08.12	Boros Á.	47° 23'	19° 07'	96
Nyergesújfalu	1931.07.05	Degen Á.	47° 46'	18° 33'	112
Soroksár-Dunaharaszti	1931.07.16	Vajda L.	47° 23'	19° 07'	96
Darány:Nagyberek	1932.07.24	Boros Á.	45° 58'	17° 35'	111
Soroksár-Dunaharaszti	1932.08.04	Pénzes A.	47° 23'	19° 07'	96
Soroksár-Dunaharaszti	1932.08.14	Degen Á.	47° 23'	19° 07'	96
Dunaszeg	1934.10.04	Polgár S.	47° 46'	17° 33'	114
Soroksár-Dunaharaszti	1938.07.	Vajda L.	47° 23'	19° 07'	96
Dunaszeg	1941.	Polgár S.	47° 46'	17° 33'	114
Soroksár-Dunaharaszti	1943.08.12	Kárpáti Z.	47° 23'	19° 07'	96
Soroksár-Dunaharaszti	1943.09.12	Jávorka S. - Keller J.	47° 23'	19° 07'	96
Soroksár-Dunaharaszti	1946.07.09	Boros Á.	47° 23'	19° 07'	96
Dunakeszi	1946.08.28	Soó R.- Kárpáti Z.	47° 37'	19° 07'	120
Soroksár-Dunaharaszti	1947.08.30	Jávorka S.	47° 23'	19° 07'	96
Soroksár-Dunaharaszti	1947.08.30	Papp J.	47° 23'	19° 07'	96
Soroksár-Dunaharaszti	1947.08.23	Papp J.	47° 23'	19° 07'	96
Soroksár-Dunaharaszti	1947.08.23	Priszter Sz.	47° 23'	19° 07'	96
Soroksár-Dunaharaszti	1947.09.09	Jávorka S. - Baksay L.	47° 23'	19° 07'	96
Dunakeszi	1948.08.28	Kárpáti Z.	47° 37'	19° 07'	120
Lipót	1951.11.19	Kovács É.	47° 52'	17° 28'	112
Lébény:Bornászpuszta	1951.11.21	Kovács É.	47° 43'	17° 22'	114
Soroksár-Dunaharaszti	1961.07.15	Papp J.	47° 23'	19° 07'	96
Sükösd	1961.10.23	Boros Á.	46° 16'	19° 00'	96
Lipót: Macska-sziget	1991.09.05	Felföldi L.	47° 52'	17° 28'	112
Dunaszeg	1995.09.03	Molnár V. A.	47° 46'	17° 33'	114
Paks	1999.07.03	Voight W.	46° 37'	18° 52'	70
Bokod	1999.08.12	Riezing N.	47° 30'	18° 15'	179
Császártöltés	1999.10.10	Kun A.	46° 25'	19° 11'	106
Sükösd	1999.10.10	Molnár V. A. - Pfeiffer N.	46° 16'	19° 00'	112
Paks	2000.07.25	Farkas S.	46° 37'	18° 52'	70
Bogyiszló	2000.09.06	Farkas S.	46° 22'	18° 49'	83

Tabe 1. Distribution of creeping marshwort (*Apium repens*) in Hungary based on herbarium data

Genetic erosion and pollution assessment methodologies: a summary of PGR Forum Workshop 5

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The main objective of PGR Forum Workshop 5 was to agree on genetic erosion and pollution assessment methodologies for European crop wild relatives (CWR). Participants considered how plant genetic erosion might be predicted and assessed using existing methodologies, while questioning its effectiveness. The workshop also considered how plant genetic pollution might be predicted and assessed. There are no established methodologies for assessing plant genetic pollution, but the threat of genetic pollution or introgression, either from genetically modified organisms (GMOs) or from conventionally bred crops, to wild species has become an increasing potential risk to the *in situ* genetic conservation of crop wild relatives.

The workshop was held at the Training Centre of the Regional Directorate for Agrarian Development, from 8 to 11 September 2004, on Terceira Island, the Autonomous Region of the Azores. It was opened with a welcoming address by the regional entities hosting the meeting and the workshop organisers, followed by a presentation of the progress reports and an update of the coming workshops. Jozef Turok from the International Plant Genetic Resources Institute (IPGRI), Rome, Italy, gave a presentation on measuring and predicting genetic change in CWR species. The joint co-coordinator of PGR Forum Work Package 5, Brian Ford-Lloyd, from the University of Birmingham, UK, presented an overview of the workshop objectives, and focusing specifically on how conservation priorities might be established for the CWR of Europe. Stefano Diulgheroff, from FAO, Rome, Italy, gave an overview on assessing and monitoring genetic erosion of CWR and local varieties using WIEWS (World Information and Early Warning System on PGR). A presentation on the political, legislative and practical aspects of *in situ* conservation following the German example in the context of genetic erosion was given by Lothar Frese from the Federal Centre for Breeding Research on Cultivated Plants (BAZ) Gene Bank, Braunschweig, Germany.

Following the presentations, three parallel working groups held discussions:
Group 1-“Political and legal issues”
Group 2-“Genetic erosion”
Group 3-“Genetic pollution”
The working groups’ results and recommendations were presented and discussed in plenary.

Day 2 was dedicated to practical aspects of measurement, monitoring and prediction of genetic erosion and pollution. Mike Wilkinson from the University of Reading, UK, talked about risk assessment and gene flow. Nigel Maxted, PGR Forum Project Coordinator, from the University of Birmingham, UK, gave a presentation reviewing approaches to the assessment and prediction of genetic erosion and genetic pollution, and introducing potential genetic erosion and pollution indicators as a means towards prioritising CWR for conservation. The applicability of the change indices as indicators of genetic erosion for the Red List assessment was presented by Caroline Pollock from the IUCN Red List Programme in Cambridge, UK. A practical example was given by François Lefèvre from the Unité de Recherches Forestières Méditerranéennes, INRA, Avignon, France, on “Genetic erosion and pollution - genetic and conservation consequences for *Populus* and other European forest species”, and Lori De Hond from the Universidad Politécnica de Madrid, Spain, on “Using populations for monitoring and prediction”. Brian Ford-Lloyd presented the genetic tools, molecular and population, for genetic assessment, followed by Jozef Turok with an introduction to the five-country GEF-funded project “*In situ* conservation of crop wild relatives through enhanced information management and field application”.

These presentations were followed by three parallel working groups:
Group 1-“Monitoring at the taxonomic level”
Group 2-“Monitoring at and around the population level”
Group 3-“Monitoring at the gene level”

Above: Participants at PGR Forum Workshop 5, Genetic Erosion and Pollution Assessment Methodologies, held at the Training Centre of the Regional Directorate for Agrarian Development on Terceira Island, the Autonomous Region of the Azores, Portugal, from 8 to 11 September 2004

Shelagh Kell

Above: Sónia Dias, co-organiser of Workshop 5, leads a working group discussion on specific needs for the assessment and prediction of genetic erosion and pollution for agricultural crop wild relatives

The working groups' results and recommendations were presented and discussed in plenary.

Presentations on case studies from the CWR list took place on day 3 of the workshop. Mike Wilkinson talked about wild brassicas. Wild forages were presented by Michael Abberton from IGER (Institute of Grassland and Environmental Research), UK. Åsmund Asdal from the Norwegian Crop Research Institute, Norway, shared his work experience with a practical example of the consequences of changes in agricultural management practices. Using the example of the old world cottons, Vojtěch Holubec from the Research Institute of Crop Production, Czech Republic, presented the situation regarding genetic erosion and extinction threat of *Gossypium* species.

The presentations were followed by three parallel working group discussions on specific needs for the assessment and prediction of genetic erosion and pollution for CWR:
Group 1-"Agricultural"
Group 2-"Horticultural"
Group 3-"Forestry"
The working groups' results and recommendations were presented and discussed in plenary. Damiano Avanzato, presented his work on "Genetic erosion of fruit varieties and their recovery from historical gardens" pointing towards practical ways to halt, and even revert, genetic erosion.

Under the session "The way forward for CWR conservation: specific proposals regarding methodologies and prospects", António Flor, from the Parque Natural das Serras d'Aire e Candeeiros (PNSAC/ICN), Portugal, shared his experiences on "Indicators for the CWR species list prioritisation", after which three parallel working groups debated:
Group 1-"The CWR species list prioritisation"
Group 2-"The hierarchy of methodologies"
Group 3-"Future demands/prospects/opportunities"

The working groups' results and recommendations were presented and discussed in plenary.

Day 4 of the workshop was dedicated to a field trip during which the participants had the opportunity to visit sites of geological and ecological interest as well as appreciating traditional agricultural systems. Prof. Eduardo Dias of the Department of Agrarian Sciences, University of Azores, led the field trip.

PGR Forum is grateful for the very generous support provided by several regional authorities for the organisation of the workshop.

Shelagh Kell

Above: Prof. Eduardo Dias of the Department of Agrarian Sciences, University of Azores, leading the PGR Forum Workshop 5 field trip

PGR Forum at the XIth OPTIMA Meeting *Lori de Hond* and *José M. Iriondo*

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The Organisation for the Phyto-Taxonomic Investigation of the Mediterranean Area (OPTIMA) held its XIth Meeting from 5 - 11 September 2004, in Belgrade, Serbia and Montenegro. PGR Forum was represented by two communications by S.L. Jury, S.P. Kell, H. Knüpffer, N. Maxted and B.V. Ford-Lloyd, and H.M. Parra, D. Draper and J.M. Iriondo.

Dr. Stephen Jury from the University of Reading, who is a member of the PGR Forum Advisory Board, presented the poster, "PGR Forum, Euro+Med PlantBase and Mansfeld's database: serving the crop wild relative user community". This poster explained the importance of crop wild relatives, and the threats posed to them across the Euro-Mediterranean region. The significance of Euro+Med PlantBase in European plant conservation activities and its use, together with the Mansfeld´s World Database of Agricultural and Horticultural Crops, as the taxonomic backbone to the PGR Forum Crop Wild Relative Information System, was explained. The poster can be viewed at: http://www.pgrforum.org/Documents/Poster_presentations/OPTIMA_Poster_FINAL.pdf

In the symposium devoted to Spatial Analysis in Mediterranean Botany, Mauricio Parra Quijano from the Universidad Politécnica de Madrid gave an oral presentation entitled, "GIS-based gap analysis for the conservation of wild relatives of crops". In this analysis the six *Lupinus* species that grow naturally in Spain were used as a model of how GIS and gap analysis can be used in the assessment of the conservation status of a crop wild relative.

Changes in grassland management and its effect on plant diversity

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Natural grasslands do not occur in Norway, and all grasslands with their species and genetic diversity have been created and developed through agriculture. This means that when agriculture ceases the diversity is lost.

The Norwegian wild flora consists of about 1800 vascular plants; among these 600-700 species are found in permanent grasslands as pastures or extensively managed meadows. About 350 species are exclusive to such biotopes. A number of these are crop species or crop related species of socio-economic value. Species and genotypes of fodder plants, such as grasses and legumes, are particularly important for Norwegian agriculture.

A short review of Norwegian agricultural history underlines the importance and value of the biological and genetic diversity developed in grasslands. Livestock was introduced 6000 years ago, open fields without trees and bushes evolved, and accordingly new species adapted to the Norwegian climate and conditions in the altered landscape. Use of manure from livestock as fertilizer in ploughed fields was introduced 2000 years ago, and through selection of adapted seeds of fodder crops, farmers increased the diversity and value of grassland species.

This means that for 6000 years species have adapted to open grasslands through grazing or mowing, or a combination of both, and a broad diversity of species and genotypes has been created. This evolution has taken place all over the country and the wide range of climatic and edaphic conditions, from south to north, from lowlands to mountain areas and from the oceanic climate to the inlands, have contributed to the extreme variation in genetic features.

This diversity is now endangered. Due to major changes in agricultural practice within farms and also the fact that large numbers of farms all over the country are shut down and abandoned, the occurrence of habitats available for the diversified flora of species adapted to grasslands has been quite dramatically reduced. The use of grasslands for grazing is decreasing and this is also the case regarding mowing of permanent grasslands for fodder production. This causes serious concerns about the destiny of species and the genetic variation within species.

“The use of grasslands for grazing is decreasing and this is also the case regarding mowing of permanent grasslands for fodder production. This causes serious concerns about the destiny of species and the genetic variation within species”

The seriousness of the process can be further illustrated with some facts. The use of outlying fields for grazing have been reduced by 50% over a 20 year period and the number of mountain farms have been reduced from approximately 45000 in 1900 to only 1000 in 2000; a reduction of more than 97% within 100 years.

There has also been a continued general decline in fields being grazed closer to the farms e.g. forest pastures and smaller and fragmented fields. EU regulations with requirements for minimum grazing period for animals may to some extent increase the use of pastures, while mowed meadows, especially in regions with steep and hilly landscapes (Fig.1), will continue to vanish.

Figures 2a and 2b show the speed of reforestation when agricultural practices are halted. Figure 2a shows the re-growth of forest in the Setesdalen valley in 1992, in a previously cultivated field with pioneer stone heaps and signs of cultivation. Small spruce trees have established and benefit from some sheep grazing. Ten years later, in 2002, spruce trees have covered the field, suppressed other plant species, concealed signs of previous agriculture and altered the view and landscape (Fig. 2b).

Two kinds of light open fields with a high diversity of grassland plant species have become widespread. These are the mowed meadows, which could be referred to as the “landscape of the scythe”, which are harvested for fodder supply in the winter, and the grazed pastures – “landscape of the muzzle”, which are used for fodder supply in the summer. In addition to these habitats with agricultural influence, grassland species can also be

Leif Hauge

Figure 1. Permanent grasslands can either be grazed or mowed. While grazing to some extent continues, partly due to EU regulations on domestic animals welfare, traditional mowing in steep landscapes with two wheel reapers is becoming very infrequent

Figures 2a and 2b. Two pictures from the Setesdalen valley showing the rapid change in vegetation and landscape when fields are abandoned. Fig. 2a (left) was taken in 1992 and shows that forest regrowth has started in a former cultivated field. Small spruce trees have established and benefit from sheep grazing. Ten years later the spruce trees significantly suppress other plant species, hide signs of previous agriculture and alter the view and landscape (Fig. 2b, right)

found in mowed marshes and on seashores; however, such fields are even more rare today.

Agricultural influence on growing conditions

Both mowing and grazing keep the landscape and the fields open and prevent forestation, but they have different impacts on the growing conditions in the field, which is also reflected in the species content of the fields.

One major difference is that animals are selective when grazing pasture, feeding only on some species, while mowing equipment cuts all species. Tasty, juicy and nutritious species are preferred by the animals, and poisonous, bad tasting, woody plants and plants with thorns are left untouched. Grazing therefore alters the species composition in favour of the plants that the grazing animals do not like.

Another difference is that because of the manuring by the animals, more plant nutrients (Nitrogen and Phosphorus) are removed from mowed fields than from grazed fields. This causes leguminous plants and less nutrient demanding plants to dominate a mowed field. Further, because of animals trampling the field, sensitive plants suffer in grazed fields and survive more easily in mowed fields. On the other hand some plant species with seeds dependent on open soil to germinate can be established in grazed fields.

In addition to these differences, the grazing and mowing practice can change conditions significantly and result in different species content. For example, the time for mowing or grazing compared to the plants' reproductive systems, and the fact that different kinds of animals have different preferences in choice of plants and remove plants in different ways.

This huge diversity in grassland habitats and agricultural practice is reflected in genetic and species diversity and the challenge is how this diversity can be conserved for the future.

Plant species in grasslands

Permanent grasslands are the only habitat for many plant species in the Nordic region. This includes endangered species, fodder species, where the plants represent a valuable gene pool for breeding, and other plants of socio-economic importance.

Examples of critically endangered and red listed plants, with grasslands as their only habitat are:

- *Melampyrum cristatum* L.
- *Ajuga reptans* L.
- *Botrychium simplex* E. Hitchc.
- *Herminum monorchis* (L.) R.Br.
- *Nigritella nigra* (L.) Reichenb.fil.
- *Liparis loeselii* (L.) L.C.M. Richard
- *Isolepis setacea* (L.) R.Br.
- *Carex extensa* Good.
- *Vulpia bromoides* (L.) S.F.Gray
- *Listera ovata* L.

Plant species used as fodder crops in Norway with an important gene pool for breeding present in permanent grasslands are:

- *Agrostis capillaris* L.
- *Poa pratensis* L.
- *Phleum commutatum* Gaud.
- *Anthoxanthum odoratum* L.
- *Dactylis glomerata* L.
- *Trifolium repens* L.

Bolette Bele

Figure 3. Increased number and size of many flowering species can be observed in the first period after agriculture ceases. A beautiful meadow with rich flowering of *Chrysanthemum leucanthemum* L. and other species can however be a sign of reforestation in the near future

- *Festuca rubra* L.
- *Trifolium pratense* L.
- *Festuca ovina* L.
- *Lotus corniculatus* L.

Figure 4. *Arnica montana* requires light, open areas with nutrient poor, acidic soils; such as grassland, hay meadows and pastures. There has been significant habitat loss over the last 50 years caused by change of land use, and the distribution will continue to decline if current changes in agriculture continue. Photo: *Kerstin Olsson*

The Nordic Gene Bank and the Nordic countries are currently evaluating their plant genetic resources in medicinal and aromatic plants (MAP) and considering how to proceed in a programme for conservation and extended exploitation. Extensively managed grasslands are the major habitat for several MAP species, and broad genetic variation is required for developing optimal production based on appropriate genotypes:

- *Carum carvi* L.
- *Arnica montana* L.
- *Humulus lupulus* L.
- *Angelica archangelica* ssp. *archangelica* L.
- *Veronica officinalis* L.
- *Achillea millefolium* L.
- *Allium oleraceum* L.
- *Mentha arvensis* L.
- *Artemisia absinthium* L.
- *Thymus vulgaris* L.

When agriculture ceases, increased humidity and a higher level of organic matter and nitrogen in the soil can be observed for a short period. This often causes an increase in the number and size of many flowering species (Fig. 3), while at the same time small and light demanding plants with a short life cycle suffer and disappear. New species establish and slowly suppress and displace the original grassland flora. As reforestation proceeds, all light demanding species are expelled and the genetic resources of these habitats are lost.

Changes in agriculture as a cause of reduced distribution of certain species

Within the context of the PGR Forum project, change in distribution and habitat loss of some species has been assessed. In Norway the medicinal plant *Arnica montana* (Fig. 4) has been included. The growing conditions required by *A. montana* are anthropogenic: light, open areas with nutrient poor and acidic soils. The habitats preferred are grasslands such as hay meadows, pastures and coastal heathland. There has been significant habitat loss over the last 50 years caused by change of land use. *A. montana* is now on the Norwegian Red List with status DC (declining / care demanding), and if the current changes in agriculture continue, the distribution of *A. montana* will be even more reduced.

Another example from the Norwegian flora is Mountain Everlasting, *Antennaria dioica* (Fig. 5), which can be considered as an indicator for species which, in order to maintain effective reproduction, needs to be numerous and widespread. Within the Norwegian Programme for Plant Genetic Resources, previous records of *A. dioica* were reinvestigated in 2002. It was clarified that the species had disappeared from a number of locations due to the cessation of agriculture, and in some remaining populations male individuals dominated. The remaining populations were more isolated and reintroduction of individuals is less likely.

It can therefore be assumed that a decline in population size and a change in population structure could accelerate when *A. dioica* and ecologically comparable species are no longer as numerous and widespread as they used to be.

Efforts in the Norwegian PGR Programme

Genetic diversity of fodder plants in Norway has not been fully investigated, but collection missions have been carried out and seed samples from agricultural and semi-wild populations of the most economically important crops are stored in the Nordic Gene Bank.

To prevent genetic erosion and potential extinction of the species found throughout the broad diversity of grassland habitats and associated growing conditions, the Norwegian Programme for Plant Genetic Resources is encouraging further use of selected grasslands. A pilot project includes mapping and evaluation of interesting fields in three counties. The documentation includes botanical data and edaphic and climatic growing conditions; agricultural history and management practices are also examined. Grassland fields with relevant species content representing diversity in growing conditions and geography are selected for continued cultivation. An evaluation of how existing grants and subsidies can be used to encourage continued traditional farming is being undertaken.

The pilot project will produce a handbook in evaluation of grasslands, recommendations for further farming, and the use of subsidies. The guidelines will be used to extend registration and evaluation of grasslands to the entire country in cooperation with regional and local agricultural authorities. Critically, recommendations will be given to the Ministry to adapt subsidies and regulations with the aim of maintaining Plant Genetic Resources.

Bolette Bele

Figure 5. Mountain Everlasting, *Antennaria dioica*, was previously a common plant in Norwegian pastures and grasslands. Reinvestigation of habitats after 20 years in 2002 showed that the species had disappeared from a number of locations due to the cessation of agriculture, and in some remaining populations male individuals dominated

First International Conference on Crop Wild Relative Conservation and Use

Wednesday 14th - Saturday 17th September 2005, Agrigento, Sicily, Italy

The First International Conference on Crop Wild Relative Conservation and Use will bring together the international community to address the current status and future of crop wild relatives (CWR) as a vital resource for improving agricultural production, increasing food security, and sustaining the environment. The Conference will be a landmark in PGR conservation, highlighting CWR as a critical but neglected resource. The Conference objectives are to:

Shelagh Kell

Above: Valley of the Temples, Agrigento, Sicily

- Promote the importance of wild plant species of socio-economic value to the international community
- Review the establishment of CWR inventories and establish a baseline for their conservation assessment
- Assess procedures for establishing conservation priorities for CWR
- Review the current status of information access and management for CWR
- Evaluate methodologies for *in situ* and *ex situ* CWR conservation
- Explore ways of strengthening CWR conservation and use through international and inter-agency collaboration
- Disseminate PGR Forum products to the European and global PGR community, and discuss their wider application and continued use

For further information and registration details, visit: http://www.pgrforum.org/conference.htm

4th Planta Europa Conference: PGR Forum represented *Brian Ford-Lloyd*

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The 4th European Conference on the Conservation of Wild Plants was held at the Botanical Garden of Valencia, Spain between 17th and 20th September 2004. As anticipated, it was a good opportunity to raise the profile of CWR conservation and use, and showcase PGR Forum and some of its achievements to date. A poster was presented (see http://www.pgrforum.org/Documents/Poster_presentations/Royal_Society_Poster.pdf), and at the workshop dealing with "*In situ* conservation and management", Brian Ford-Lloyd, PGR Forum Deputy Project Coordinator from the University of Birmingham, UK gave a talk entitled "European crop wild relative diversity and conservation". Other workshops dealt with "Selection and designation of conservation areas", "Impacts on wild flora and their correction, sustainable development" and "Education, public awareness for plant conservation". A workshop on "*Ex situ* conservation / *Ex situ* and *in situ* research" was also organised by PGR Forum Partner, José Iriondo, from the Universidad Politécnica de Madrid.

The conference was informative and stimulating, not least because of the considerable interest shown in crop wild relatives. This was strongly underpinned by the success of PGR Forum Advisory Board member Wendy Strahm, Plants Officer of the IUCN Species Survival Programme, in gaining support for the initiation of the IUCN/SSC Crop Wild Relative Specialist Group.

This was the first Planta Europa meeting to be held since the publication of the European Plant Conservation Strategy which followed from the work of the Planta Europa conference held in 2001. Support from Governments and NGOs alike, and endorsement by the Parties to the CBD as well as the Government of the Council of Europe demonstrates the foundation that has been laid by Planta Europa in implementing the Global Strategy for Plant Conservation (GSPC). It followed therefore that the focus of the 4th Conference in Valencia should be the implementation of the GSPC, demonstration of the progress made towards meeting the targets, and debate on the best practice for conserving wild plants in Europe. At the end of the conference, participants were asked to focus on a critical re-evaluation of the targets agreed in 2001 by Planta Europa to be met by the 2007 conference. It seems likely that revision of some of the targets will result, and some of these are relevant to PGR Forum. Some of the final discussion revolved around the following issues:

- The need for a clearing house mechanism and information exchange on European plant conservation work
- The need for a wake-up call to politicians and the media for plant conservation
- Species management / recovery plans implemented for at least 60% of species listed in the Bern Convention (including crop wild relatives)
- The need for a European Plant Red Data list

At the final conference dinner there were many awards made to individuals who had made significant contributions to plant conservation. David Bellamy, a popular figure in conservation and campaigning was one, and his after dinner speech was significant in highlighting the serious demise of plant biology in higher education in the UK and Europe. Another receiving an award and deserving our congratulations was a member of the PGR Forum Stakeholder Panel, Professor Vernon Heywood.

Novel sewerage treatment technologies: a new group of potential crop plants emerges to be conserved *in situ*

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There is increasing concern about waste water treatment generally, but especially in the Central European countries newly accessioned to the European Union. Novel technologies are emerging that are more effective, releasing more completely purified water at the outlet. These "third generation technologies" apply whole ecosystems in the purification process. These include aquatic plants, microorganisms associated with their root systems and many animal species. Part of such a system is shown in Figure 1.

An enterprise working on the design and operation of novel technology sewerage treatment plants, contacted the Institute for Agrobotany to cooperate in the collection and study of plant species suitable for their technologies. Our partner, the Körte-Organica Rt has been developing "Organica Technology" for the past six years and also holds the license for the "Lake Restorer technology". Both of these technologies are based on the use of ecological communities associated with the roots of aquatic plants.

In the Organica treatment plant the process takes place in aerated sewerage tanks, covered by a climate controlled greenhouse. The "Lake Restorer" technology is applied in ponds and lakes polluted by sewerage.

We have been collecting, investigating and propagating plant species considered suitable for such technologies for five years. During our cooperation, four sewerage treatment plants have been completed; three in Hungary, one in Poland, and several more have been under construction this year. In the first two years of our collaboration, the emphasis was on investigating the suitability of different plant species for the technology. The selection criteria were:

- Water tolerance
- Tolerance to maximum summer greenhouse temperatures (up to 39 C)
- Tolerance to low winter temperatures (+6 C)
- Ease of propagation
- Aesthetical value
- Leaf retention during winter

We have tested over 120 plant species, both native and tropical. Three to five plant specimens of each species were grown in the greenhouse in pots, and kept in 5-10 cm of water. The maximum summer temperatures often reached 36-38 C, while in winter the greenhouse was kept frost-free by slight heating. Winter temperatures ranged between +3 and 15 C. The root development of white poplar (*Populus alba*), after growing in water for two months, can be seen in Figure 2.

Certain species of the following genera met the technology requirements during the preliminary experiments: *Acorus* L., *Ajuga* L., *Alisma* L., *Aster* L., *Ballota* L., *Butomus* L., *Calystegia* R.Br., *Carex* L., *Chrysanthemum* L., *Cornus* L., *Eleocharis* R.Br., *Epilobium* L., *Euonymus* L., *Geum* L., *Glyceria* R.Br., *Hemerocallis* L., *Inula* L., *Iris* L., *Juncus* L., *Lycopus* L., *Lysimachia* L., *Mentha* L., *Plantago* L., *Populus* L., *Potentilla* L., *Prunella* L., *Ranunculus* L., *Rorippa* Scop., *Sagittaria* L., *Salix* L., *Schoenoplectus* (Rchb.) Palla, *Scrophularia* L., *Sium* L., *Sparganium* L., *Symphytum* L., *Taraxacum* Weber, *Tussilago* L., *Typha* L., *Valeriana* L., *Veronica* L. and *Viola* L..

Many of these species are common, but the investigation and conservation of their genetic diversity is nonetheless needed. There are also several endangered species included in this list. As nature conservationists generally consider them only at taxo-

Figure 1. A view of the "Organica Technology" system. The plants are sitting on a mesh, placed at the water level in the aerated sewerage tank. Their roots submerge in the sewerage, being under treatment, providing large surfaces for microflora development

nomic level, investigation of genetic diversity is important. However, the largest part of the list consists of species that are not common, but not rare enough to treat them directly by nature conservation measures. These taxa would be the most important target for our team, because of the limited knowledge both of the present sites, and genetic diversity of the existing populations.

Since completion of the three sewerage treatment plants with "Organica Technology", the plant species applied have been monitored for survival, plant health, vigour and leaf retention. The first treatment plant has been working for 3 ½ years, the second for 2 years, and the third for 1 ½ years. According to

Figure 2. Root development of white poplar (*Populus alba* L.).seedlings during two months of submersion in water. The author evaluated the root development of about 120 species this way

combined results of the three sites, the following species proved to be useful in the present forms of “Organica Technology”: *Acorus calamus* L., *Carex acutiformis* Ehrh., *C. pendula* Huds., *C. riparia* Curtis, *Epilobium hirsutum* L., *Glyceria maxima* (Hartm.) Holmb., *Iris pseudacorus* L., *Juncus effuses* L., *J. inflexus* L., *Lythrum salicaria* L., *Mentha aquatica* L., *Schoenoplectus lacustris* (L.) Palla, *Salix cinerea* L., *Typha latifolia* L. and *Typhoides arundinacea* (L.) Moench. “Organica Technology” is under continuous improvement, with our contribution, partly to make it more “friendly” for plants. The “Restorers” are being developed for diverse environments, therefore it is foreseen that a wide range of plants will be applied in future.

The “Restorer technology” yet to be tested, but it is important to note that only winter hardy species can be used in this technology, so the role of native species is becoming more important.

It is concluded that there are numerous species of importance for novel sewerage treatment technologies, and probably even more that can be useful as the technologies evolve. The *in situ* preservation of the useful, and potentially useful species is important, especially as aquatic biotopes are threatened by climate change and human influences. Thus the *in situ* conservation of aquatic and marshy plant species would require the conservation of aquatic habitats. For the *in situ* conservation of plant species useful for “Organica Technology” and “Restorer” technologies, the following steps are proposed:

- Determine the target species
- Locate existing populations
- Assess the within- and between-population genetic variation for selected traits in Central Europe
- Evaluate the variation of characters associated with the suitability for the technology.

As the knowledge and needs of our society grow and diversify, new groups of plants become potential as crops. Until now, only a small group of aquatic plants have been cultivated as ornamentals, but the development of novel sewerage treatment technologies draws our attention to a much larger groups of aquatic plants to be studied, conserved and utilised.

Genetic variation in wild Brassicas in England and Wales

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There are 8 species of the genus *Brassica* L. (cabbages) found in the UK (Stace, 1997). Many are important crops and the others are crop wild relatives. *B. napus* L. is grown pan-temperate as oil seed rape, *B. oleracea* L. is the most widely grown food brassica and is cultivated as cabbage, cauliflower, broccoli, sprouts, etc., *B. rapa* L. is cultivated as food and fodder turnip and *B. juncea* (L.) Czern is widely cultivated for its mustard seed. These *Brassica* species have been so intensively bred and cultivated for such a long time that their genetic base has narrowed. Crop wild relatives of these species undoubtedly contain a much larger gene pool that can and is being used to enrich crop diversity. Specifically they may also have genes that can confer resistance to disease and pests that do not exist in the cultivated forms. This reinforces the critical need to understand and conserve our natural genetic resources. However, to effectively achieve this goal it is necessary to understand the natural distribution of such diversity.

As a first step toward understanding the natural distribution of genetic diversity of *Brassica* species in Britain the University of Birmingham undertook a study of the genetic variation in wild *Brassica* species (*B. nigra* (L.) W.D.J. Koch, *B. oleracea* and *B. rapa*) in England and Wales using AFLP (amplified fragment length polymorphism). AFLP technology (Vos *et al*., 1995) gives highly informative genetic fingerprints and requires no prior knowledge of the target taxon’s DNA sequence. Instead, generic primers are used to amplify restricted fragments and so this technique can be applied to any species with a relatively small amount of trial and error to identify the most useful combination of primer pairs.

The AFLP results were statistically analysed to determine whether there were any correlations between genetic dis-

Sarah Watson-Jones

Figure 1. *Brassica nigra*

Figure 2. *Brassica oleracea*

"AFLP results were statistically analysed to determine whether there were any correlations between genetic distance and ecogeographic distance between populations for each species"

tance and ecogeographic distance between populations for each species. Levels of within population genetic diversity were compared with geographic distance and 27 environmental parameters, such as soil type (inc. pH, soil structure, organic content, etc.), rainfall and temperature to determine whether any such correlations exist.

Species involved in the study

Brassica nigra (Figure 1) is a British native fairly widespread throughout England with some records for Wales and South Scotland. It was once a major crop in temperate regions but since the 1950s it has largely been replaced by *B. juncea* (Westman and Kresovich, 1999). Pollination in natural populations is mainly insect-mediated, but it is unclear to what degree the species is inbreeding. Levels of self-fertilisation in natural populations are unknown but there are suggestions that it does occur to varying degrees. At present there is only one *ex situ* UK seed accession, collected from Dorset and held in the UK in the Millennium Seed Bank (Royal Botanic Gardens, Kew).

Brassica oleracea (Figure 2) is listed as nationally scarce in Britain by Richards (1994). Wild populations are coastal and restricted to southern England, north and south Wales and Yorkshire. It is believed to have been introduced to Britain by the Romans and has since naturalised with the first record for a wild British population being recorded on the cliffs at Dover in 1551 (Mitchell, 1976). *B. oleracea* is self-incompatible and so an obligate outbreeder, pollinated by insects. Nine UK populations are represented as seed accessions in the gene bank at Horticulture Research International, Warwick and seventeen populations are held in the Millennium Seed Bank (Royal Botanic Gardens, Kew). In addition, several populations are known to exist in protected coastal areas, although these reserves are not managed specifically for the conservation of *B. oleracea*.

Brassica rapa is widespread in England, and is also more common in Wales, Scotland and Ireland than *B. nigra* but is generally believed to have been introduced to the UK (Preston, Pearman and Dines, 2002). *B. rapa* is also self-incompatible and pollinated by insects. There are no UK populations currently represented *ex situ* collections and there are no populations thought to be present in UK protection areas.

Methodology

In total, fifteen populations of *B. nigra*, eight populations of *B. oleracea* and nine populations of *B. rapa* were sampled; see Figure 3 for distribution map of sample populations. These populations were chosen to represent the broadest ecogeographic distribution based on comprehensive records from herbaria, vice county records and web-based accession databases. Sampling involved the collection of leaf material from up to 30 different plants per population. Accurate location was recorded using a global positioning system and other field notes were made such as population area, habitat, management, associated vegetation and so on. Further ecogeographic information concerning each population was gained using information from NATMAP vector (NSRI, 2002). Figure 4 shows the populations plotted over the soil map and Figure 5 show a detail of three populations to give an indication of the achievable resolution. Once the soil type is known the soil legend provides data concerning many details of the soil content and associated land uses. The same software also has maps of rainfall and temperature, which were used in the same way.

	Primer combination	
	Primer 1 (P1) - labelled with blue dye	Primer 2 (P2) - labelled with green dye
*Eco*RI	5'-GACTGCGTACCAATTCACA-3'	5'-GACTGCGTACCAATTCACG-3'
*Mse*I	5'-GATGAGTCCTGAGTAACTT-3'	5'-GATGAGTCCTGAGTAACAC-3'

Table 1. Sequences of the two selective primers pairs that were used

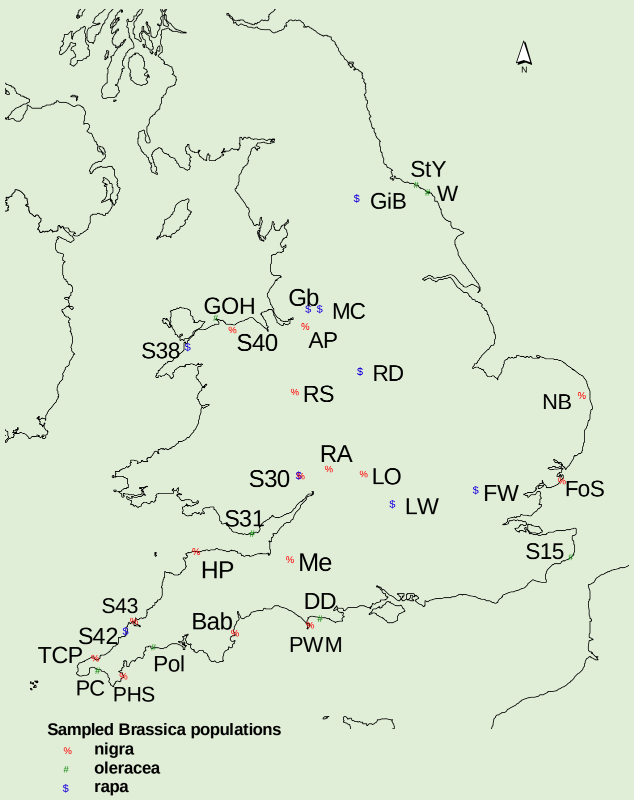

Figure 3. Map showing locations of populations sampled for the study

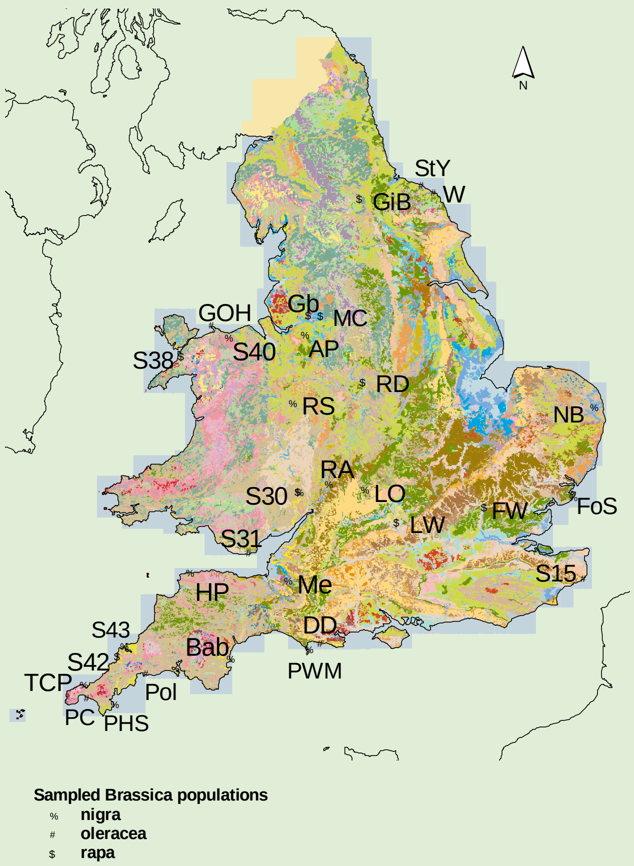

Figure 4. Sampled populations overlaid on a soil map of England and Wales

"the full range of genetic diversity to be found in *B. nigra* and *B. rapa* cannot be represented by one population....therefore, to complete a core collection for these two species it would be necessary to collect from a number of populations from a broad geographic area"

AFLP

DNA was extracted from the dried leaf material using a modified CTAB DNA extraction procedure adapted from Gawel and Jarret (1991). The protocol for the AFLP analysis follows that of Perkin-Elmer-Applied-Biosystems (1997) with very minor modifications and is based on the protocol described by Vos *et al.* (1995). Two selective primer pairs were selected (see Table 1) on the basis of showing clear polymorphism across all three species.

The amplified products were run on an ABI Prism 3700 capillary sequencer. The resultant electrophoregrams were analysed using ABI Genotyper 3.7 software. The data produced were also assessed manually to ensure that bands had been scored accurately and to prevent false identification of polymorphisms.

Results

B. nigra

Populations were relatively highly differentiated, Fst = 0.3615, P = 0.05, which can also been seen in the PCoA (principal coordinate analysis) plot (Figure 6), where individuals in populations are grouped, despite some overlap between populations. Genetic distance and geographic distance between populations were seen to be significantly correlated (P = 0.05). A stepwise multiple regression analysis was carried out to see if any abiotic factors could account for the different levels of genetic diversity of the populations but all variables were excluded.

B. oleracea

Populations did not highly differentiate despite a significant Fst value, Fst = 0.2257, P = 0.05. The PCoA plot (Figure 7) shows that most individuals are spread across all axes, with population

Figure 5. Detail showing sampled populations overlaid on the soil map of England and Wales

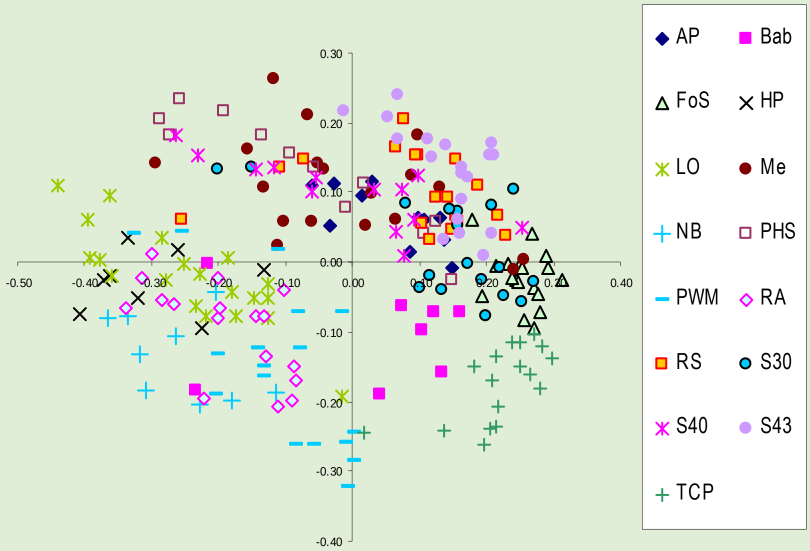

Figure 6. Principal coordinate analysis of genetic diversity for *B. nigra* populations based on simple matching coefficient, coordinates 1 vs. 2

S15 being most tightly grouped. This population was collected from Langdon Cliffs in Kent, close to the originally described population of wild *B. oleracea* in Britain. Genetic distance between populations was not correlated to geographic distance, P = 0.36. However, there was a significant correlation between pH and % polymorphism within populations, the higher the pH the lower the polymorphism.

B. rapa
Populations were significantly differentiated, Fst = 0.2168, P = 0.05. The PCoA plot (Figure 8) shows that samples from individual populations group together. Genetic distance was significantly correlated to geographic distance, P = 0.01. An R^2 value of 0.3949 shows that geographic distance accounted for almost 40% of the genetic distance that was observed, perhaps this could be a consequence of seed and pollen dispersal along water courses. Stepwise multiple regression analysis showed a significant correlation between % coarse sand particles in the soil and % polymorphism within populations, P = 0.022. All other variables were excluded.

Discussion
The results indicate that the full range of genetic diversity in *B. nigra* and *B. rapa* cannot be represented by conservation of a single population. The range of genetic diversity observed spreads across a number of populations and thus it would be necessary to conserve multiple populations from disperse eco-geographic conditions to ensure conservation of the full range of genetic diversity. These two species are both fairly widespread in England and, to a lesser extent, in Wales and neither is under any immediate and obvious threat. However, recent studies have shown that there is a likelihood of hybridisation between *B. rapa* and the closely related *B. napus* (Wilkinson *et al.*, 2003), and the potential introduction of genetically modified *B. napus* to the UK means *B. rapa* should be considered a priority.

Conversely *B. oleracea*, the most well-studied and of the three the most well-represented in gene banks and most commonly found in protected areas, could be effectively represented sampling or protecting far fewer populations. Genetic diversity in most of the populations studied was spread fairly evenly across the axes of the PCoA plot. Only one population was more tightly grouped and not spread across all axes, S15, which came from Kent and was geographically closest to the originally described population in the 16th century. It is widely speculated that *B. oleracea* populations in Britain are cultivar escapes that quickly revert back to their wild form (Wilkinson, Rich, Raven, pers. comm.). This may explain the lack of structure regarding genetic to geographic relatedness.

"recent studies have shown that there is a likelihood of hybridisation between *B. rapa* and the closely related *B. napus* .... with the potential introduction of genetically modified *B. napus* to the UK, this may be cause for concern"

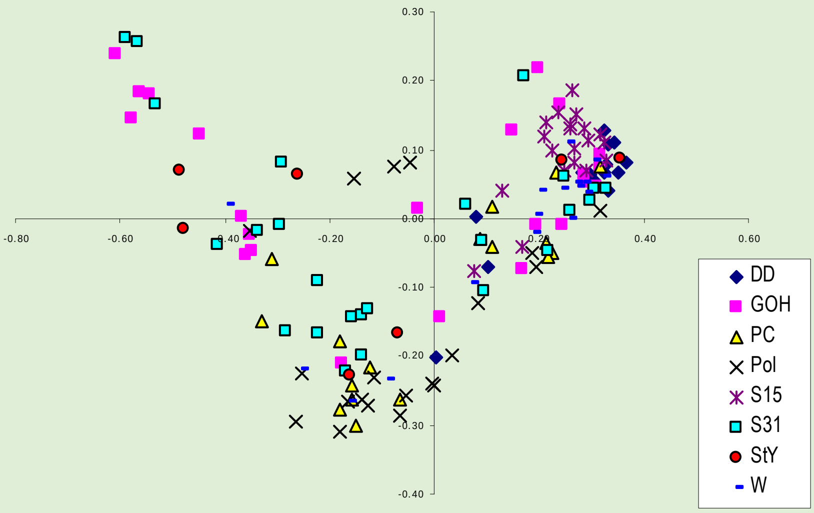

Figure 7. Principal coordinate analysis of genetic diversity for *B. oleracea* populations based on simple matching coefficient, coordinates 1 vs. 2

Environmental characteristics appear to play a limited role in partitioning *Brassica* diversity. The only statistically significant correlations observed for all the edaphic and climatic factors considered were between levels of within population polymorphism and pH for *B. oleracea* and within population polymorphism and percentage of coarse sand particles in the soil for *B. rapa*. The higher the pH the lower the polymorphism recorded for *B. oleracea* and the higher the percentage of coarse sand the lower the polymorphism in *B. rapa*. This requires further investigation to draw any reliable conclusions but it is possible that higher levels of stress, i.e. pH at the higher end of most plants normal growth range or less available water due to coarser soil, are reducing levels of polymorphism perhaps because a more specialist adaptation is necessary.

Overall, the results indicate that there is sufficient *B. oleracea* already conserved *ex situ* but that *B. nigra* and *B. rapa* should be better represented in *ex situ* collections taking samples from a broad ecogeographic distribution. In terms of *in situ* conservation of wild Brassicas the situation is less clear cut. It would be difficult to identify any particular populations as being centres of genetic diversity within England and Wales for these three species where genetic reserves should be established. Also given that none of the species are under any immediate obvious threat it is unlikely with the limited conservation resources available that they would receive priority over the more threatened species in Britain. However, as the genus is a priority UK genetic resource it is recommended that regular monitoring of populations is established to allow reassessment of the situation should these species prove to be threatened in terms of population and habitat loss or potential gene flow from cultivated varieties in the future.

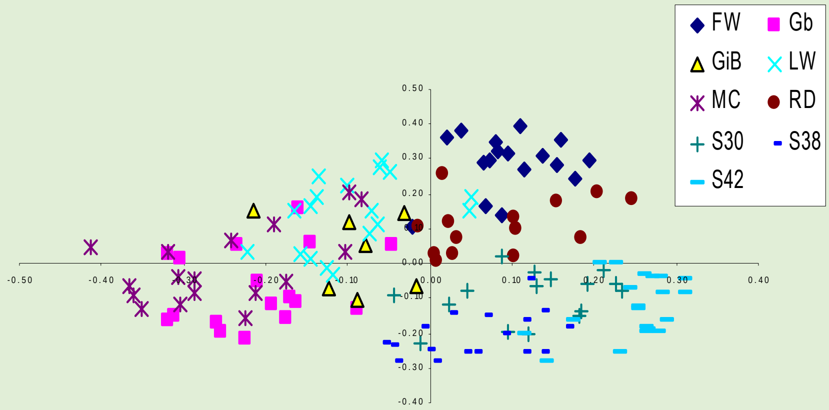

Figure 8. Principal coordinate analysis of genetic diversity for *B. rapa* populations using simple matching coefficient, coordinates 1 vs 2

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