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Natural soil spore banks – can they be used to retrieve lost ferns?

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Some fern species form spore banks – reservoirs in the soil of viable spores which remain dormant while buried but germinate in light if brought to the surface. The recently discovered characteristics of these spore banks are described. Enough is now known to suggest that they might have a role in the conservation of endangered fern species as alternatives to *ex situ* collections of sporophytes, gametophytes and spores, the relative merits of which are also considered. Mature sporophytes of several British species have now been raised from natural spore banks in soil samples; if this proves to be possible also for endangered species, some interesting options become available. The possibilities are discussed of augmenting surviving populations and increasing their genetic diversity, even perhaps of retrieving lost populations, by reintroduction of spore bank-derived plants or by stimulating regeneration from spore banks *in situ*. Botanic gardens are well placed to provide the further research, the regular monitoring of endangered populations, and the taxonomic and horticultural support required to realise these possibilities.

Keywords ferns; soil banks; spore banks; conservation; reintroduction

Introduction

Ferns make an important contribution to the world's plant diversity. They form the second largest group of vascular plants and they are a significant, sometimes dominant, component of many plant communities. Furthermore, they are rich in secondary compounds (Soeder, 1985), some of which might provide medicinal drugs, insecticides or antibiotics. It is therefore, a matter for concern that more than 10% of the 12 000 species of ferns are threatened with extinction (Kramer and Green, 1990), largely because of habitat destruction by man. There is a need to conserve endangered species. The primary need is to protect habitats for *in situ* fern conservation, but there is also a need for supplementary, and complementary, *ex situ* conservation to provide plants for reintroduction to the wild (Maunder, 1992) or for living collections to be used for research, education or horticulture.

Before considering the alternative sources of material for *ex situ* conservation, it is necessary to review the three distinct stages of the typical homosporous fern life cycle (for details of the complete life cycle, see Bold *et al.*, 1987).

The sporophyte is the familiar fern 'plant' a relatively large long-lived perennial, sometimes reproducing asexually as well as sexually.

The spore is the small (<0.1 mm) dispersal unit produced by meiosis in very large numbers by the sporophyte: dormant within a thick protective wall when dry, it germinates in moist conditions to produce the gametophyte.

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The gametophyte is a relatively small (usually <1 cm), simple, shortlived (1–2 years), independant plant which establishes at the new site determined by wind dispersal of the spore and then produces gametes: gamete fusion ultimately produces a new sporophyte at the same site.

Thus, unlike the seed plants, homosporous ferns have two, very different, free-living stages, both of which have to succeed at the same site, while the dormant dispersal unit is not a relatively large seed containing an embryo within maternal tissue but a single-celled spore. In the relatively few heterosporous ferns, such as *Azolla*, *Salvinia* and *Pilularia*, the gametophyte is much reduced and the spores are produced and dispersed within a seed-sized, highly durable, sporocarp. Although the gametophytes do not live for more than a few days, the sporophytes offer the same opportunities for conservation as in homosporous species (see below). Similarly, many of the characteristics of homosporous fern spores which are significant in relation to conservation (see below) are also shared by the heterosporous sporocarps: they are not produced in such large numbers nor are they widely dispersed by wind, but they are extremely durable and can remain viable in the mud in which the parent sporophytes grew or in dry storage at room temperature for many years (Sussman, 1965; Lloyd and Klekowski, 1970). However, each sporocarp contains many megaspores and microspores and thus can contain within it the potential for genetic variation, unlike a single spore of a homosporous species.

Sources of material

There are four ways of bringing a homosporous fern species into an *ex situ* collection and maintaining it there.

As sporophytes:

These have some advantages: they are identifiable (fern taxonomy is based mainly on the sporophyte) and, in a growing collection, most of them are long-lived (at least decades) and usually provide an annual source of fresh spores of known parentage. Unlike the seeds of angiosperms, which are formed immediately after fertilization, spores produced from identified fern sporophytes can not be contaminated by hybridisation because fertilisation does not occur until after spore release. Sometimes sporophytes are the only available source of material and provide the only means of avoiding extinction. Such cases will include newly-formed sterile hybrids. After restoration of fertility through chromosome doubling, sterile hybrids have played a central role in the past evolution of many fern species and, if conservation is to be concerned with preserving the evolutionary process and potential as well as the individual products of evolution (some of which are proceeding irreversibly towards extinction), there will be circumstances in which conserving sterile hybrids is desirable. Sporophyte collections are also necessary to provide visual displays for education about fern conservation. However, many sporophytes are difficult to propagate clonally by horticultural methods, collections of the larger species are likely to be limited by space and some species are difficult or expensive to maintain because they require special growth conditions. In addition, although some clonal sporophytes are thought to live for several hundred years (Sheffield, Wolf and Haufler, 1989), the growing tips might be subject to genetic change due to accumulating mutations. Furthermore, collecting material initially from the wild as sporophytes is liable to further deplete endangered populations, and mature sporo-

phytes are difficult to transport successfully because they are bulky, vulnerable *in transit* and sometimes difficult to re-establish in cultivation.

As gametophytes:

It is seldom advantageous to use gametophytes. They are difficult to find in the wild and rarely identifiable to species. In culture they are usually relatively short-lived (up to 2 years), although clonal propagation can sometimes be induced. Only in a few, highly specialized species with 'independent' gametophytes (Farrar, 1985) is a collection of this phase of the life cycle likely to provide a practical means of *ex situ* conservation. In these ferns, including *Trichomanes* and *Vittaria* species, the branching ribbon-like perennial gametophytes form dense mats and also reproduce asexually by means of dispersible gemmae. In this way they can spread and maintain themselves in the wild beyond the ecological and geographical range of the sporophytes. It is possible, therefore, that these permanent gametophyte clones contain genotypes not represented in the sporophytes of sexual populations. Maintaining conservation collections of these independent gametophytes might, therefore, preserve greater genetic diversity than exists within the sporophytes. The gametophytes are restricted to a specialized habitat on moist, shaded, rock surfaces but can be locally abundant and then relatively easy to locate, identify and collect. Each collection can then be separately maintained as a clone on a standard substrate in small dishes, minimizing the demand for space.

As newly-released spores:

As compact, dormant, durable, dispersal units, fern spores are analogous within the life-cycle to angiosperm seeds. They also have some of the same advantages as sources of material for *ex situ* conservation. They are abundantly produced by most species (up to 750 million on a single frond) and compact, with the genetic potential of each individual contained within a single cell. A representative sample of a population can, therefore, be collected easily and transported, and the genetic diversity of a species throughout its range stored in a very small space. The spores of most species tested are long-lived (up to 70 y) in conventional storage conditions (4° C, air-dry or over a desiccant) and some last almost as long at room temperature (Lloyd and Klekowski, 1970; Dyer, 1979; Page, 1979; Windham and Haufler, 1986; Windham *et al.*, 1986) spores are easily cultured to produce gametophytes and then sporophytes when required for research, re-introduction, education or release into horticulture. During spore storage, the genetic diversity is protected from the selection processes that operate when sporophytes or gametophytes are grown in artificial *ex situ* culture conditions. Also, the spores probably maintain their genetic integrity for many years and at least as long as long-lived or clonal sporophytes do. Although spores might be subject to mutation during ageing in storage, as has been observed in seeds (Levin, 1990), spores retain their viability, and perhaps their genotype, longer when they are stored in the hydrated state (Lindsay, Williams and Dyer, 1993). However, spore collection and storage can present difficulties. In some hybrids and polyploids, no viable spores are produced. In many species, spore production is restricted to a short season and in some species, annual spore production is unpredictably suppressed in particular years and localities. This makes collection especially difficult in remote areas. Furthermore, the spores of a minority of species, including the chlorophyllous spores of the genera *Osmunda* and *Cyathea*, are short-lived (a few weeks) in standard storage conditions (Lloyd and Klekowski, 1970). Although

spore collection is likely to be less damaging than removing sporophytes, there might even be instances where removal of part of the annual spore production in a small species might critically affect the reproduction of the few remaining individuals in an endangered population.

Despite the disadvantages, this approach, like the two preceding ones, can be successfully employed with appropriate species and all three are being developed in a cooperative programme currently involving C.N. Page and D.G. Mann at the Royal Botanic Garden. Edinburgh (RBGE) and myself at Edinburgh University (Page *et al.*, 1992). Glasshouse and outdoor sporophyte collections are being established in Edinburgh and in outlying gardens in Scotland and Cornwall. We are also developing an *ex situ* spore storage facility using improved storage methods (Lindsay *et al.*, 1992).

As natural spore banks:

This is an entirely new approach and more research is needed before its value for conservation can be fully assessed. However, results to date indicate that natural soil spore banks have considerable unrealized potential as a source of conservation material. The purpose of this paper is to discuss this potential in the light of what is currently known of the characteristics of spore banks

Characteristics of soil spore banks

Spore banks, analogous to seed banks, occur when spores enter the soil where they remain viable but dormant. Evidence that the soil contains reservoirs of viable fern spores has been accumulating since 1974 but only recently has there been any attempt to systematically study these spore banks (Lindsay and Dyer, 1990; Dyer and Lindsay, 1992).

Most of the available information has been obtained by taking soil samples at several different depths from a variety of sites, and then culturing them to induce gametophyte development. Most species require a light stimulus for spore germination, so viable spores on the surface of the sample will develop when cultured in light. There will be other viable spores below the surface but they will not respond and will be undetected. Species like *Botrychium* and *Ophioglossum* that germinate and form gametophytes within the soil will also not be recorded. The number of gametophytes on the surface thus gives only a relative measure of the number of viable spores in the soil. Moreover, only those spores capable of germinating on the soil substrate within which they were sampled will be recorded. Thus, the absence of gametophytes on cultured samples of *Sphagnum* peat in which fern spores were visible (Dyer and Lindsay, 1992) might have been due to the inhibitory effect of live *Sphagnum* shoots on fern spore germination (Clymo and Duckett, 1986). Identification to species is rarely possible but gametophytes are recorded as having trichomes (+T) or not (−T). The presence or absence of these hairs is a constant species characteristic. Presence of both types of gametophyte within a culture thus reveals that at least 2 species are present in the sample. Within many cultures, differences of trichome morphology and other gametophyte characteristics such as cell size and prothallus shape suggest that there are frequently several species present. However, gametophyte taxonomy is not yet developed sufficiently to make further analysis possible using morphological characters. Only after further culture for several months is positive identification possible and then only for those gametophytes that form

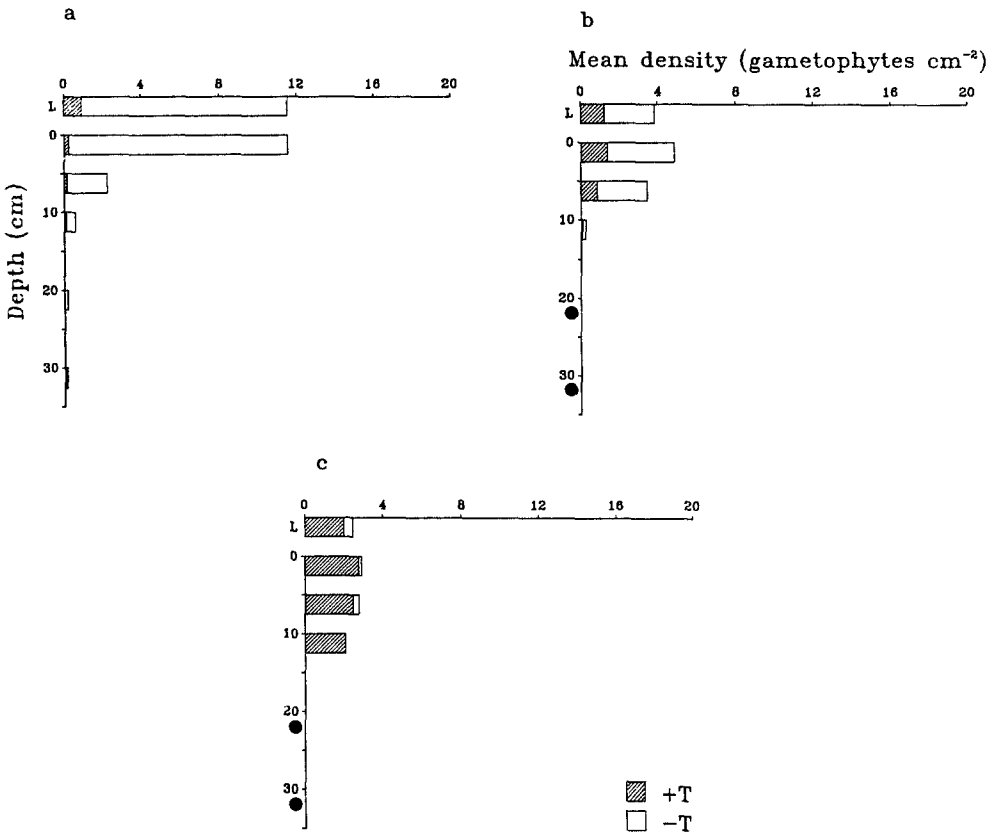

Figure 1. Spore bank profiles for three sample sites in a 100–110 year old mixed pine and deciduous woodland in Area 57, Durham Division, Duke Forest, Durham, North Carolina, USA (September 3, 1987). a. Samples taken immediately beneath a sporing plant of *Athyrium asplenioides* (Michaux) A.A. Eaton (−T). The same plant was also the nearest fern sporophyte to sample sites b and c. The nearest plant of *Polystichum acrostichoides* (Michaux) Schott (+T), the only other fern species with surficial gametophytes within 100 m, was about 6 m from a. b. Samples taken 2 m from a. c. Samples taken 10 m from site a., 8 m from site b., in line with both. At a greater distance were occasional scattered individuals of *Polystichum acrostichoides*. The profiles show that an enlarged spore bank dominated by species with naked (−T) gametophytes extended for at least 2 m but less than 10 m from the presumed source. At least two species are present at all three sites. As in all the subsequent profiles, L = Litter and the soil depth scale stops 2.5 cm below the deepest sample obtained. ● indicates depths at which samples were taken but no viable spores detected. The gametophyte frequencies were calculated from the mean number of gametophytes growing on the surface of soil samples in 3 (2 for Fig. 6) replicate 5 cm Petri dishes (soil surface area = 19.6 cm^2) or 3.6 cm × 3.6 cm seed tray compartments (area = 13.0n cm).

sporophytes. Nevertheless, despite its serious limitations, this simple technique has yielded much new information about the characteristics of fern soil spore banks. These characteristics are discussed in detail elsewhere (Lindsay and Dyer, 1990; Dyer and Lindsay, 1992) and are summarized below.

Widespread occurrence

Fern spore banks have been found in Europe, N America, Mexico, Australia, New Zealand and Malaysia. They have been found at sites in every habitat type examined, including ancient deciduous and coniferous forest; successional stages of secondary forest; arable fields, abandoned fields and permanent pasture; heather moorland and *Sphagnum* bog; creek-bank alluvial sand and waterlogged fen: coastal caves, sea cliffs and exposed inland rock-faces; and urban parks and wasteground. Within this list, spore banks occurred at almost every site sampled.

Two or more species in each spore bank

Almost without exception, spore banks contained two or more species, even when samples were taken from soil immediately beneath fronds releasing spores (Figs. 1 to 3, 5 to 6). This is even true of samples taken on a sea cliff, on the Isle of Iona off the west coast of Scotland, which faced directly into the prevailing wind off the Atlantic. There was a population of *Asplenium marinum* L. on the cliff which provided a possible source for the gametophytes without trichomes that grew on the cultured samples. However, the cultures also contained a few gametophytes with trichomes despite the fact that the nearest sporophytes of species with that characteristic (*Dryopteris dilatata* (Hoffm.) A. Gray) were at least 100 m inland from the sample site, behind the cliff and usually downwind from it.

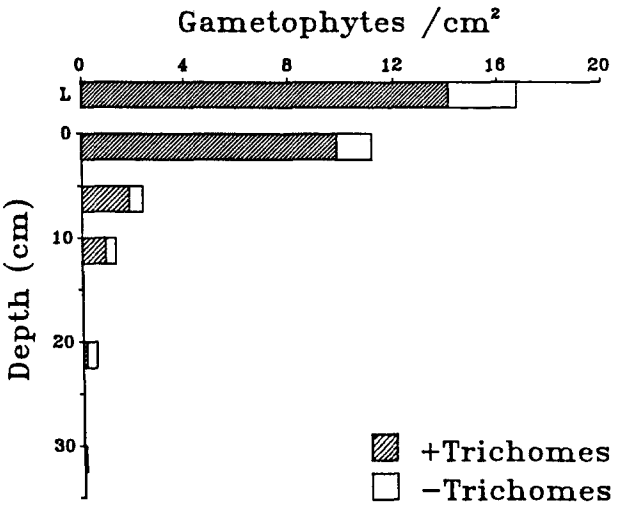

Figure 2. Spore bank profile for a sample site in the same woodland as in Fig. 1. (September 1987). The samples were taken immediately beneath a fertile plant of *Polystichum acrostichoides* (+T) and 2 m from plants of *Athyrium asplenioides* (−T). There were other plants of both species approximately 20 m from this site along the same creek bank and occasional plants of *P. acrostichoides* scattered through the wood but no other species with surficial gametophytes for more than 100 m. The profile shows that the size of the spore bank declined with depth but the proportions of naked and trichomatous gametophytes remained approximately constant.

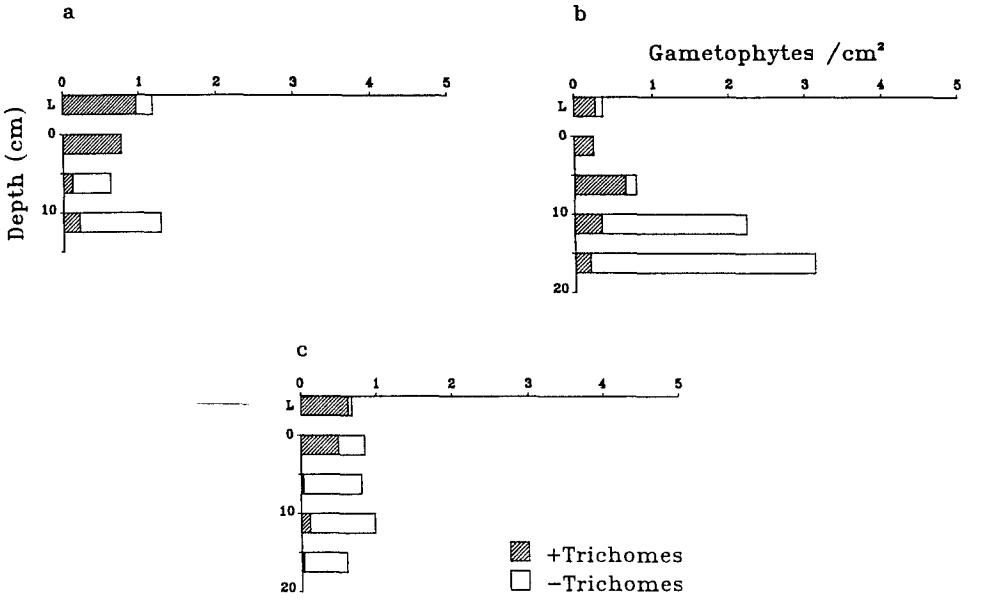

Figure 3. Spore bank profiles for sample sites in a population of bracken. *Pteridium aquilinum* (L.) Kuhn. subsp. *aquilinum* (−T) on an open hillside near Edinburgh, Scotland (August 8 and December 22, 1989). The bracken fronds were moderately fertile but for several years previously had been sterile. There were a few small, sterile plants of *Blechnum spicant* (L.) Roth (−T) scattered among the bracken, but no other ferns within 800 m. Sampling below 15 cm (a.) and 20 cm (b.) was prevented by a dense layer of small stones. Profiles a. and b. were obtained on the same date (8.8.89) from similar sites about 25 m apart; profile c. was obtained four months later (22.12.89), within 25 cm of b. Profile b. shows the spore bank increasing in size with depth; all three show a marked change in species composition at 5–10 cm due mainly to an increase in the number of (−T) gametophytes. Many of the gametophytes on the 10 cm and 15 cm samples had the characteristics of *Blechnum spicant*; none was identified as bracken.

Size and species composition vary with location and distance from source.

As might be expected, the largest spore banks, giving the largest numbers of gametophytes in culture, occurred in samples taken immediately beneath sporing fronds in forests (Fig. 1). At a distance of 2 m from the nearest source, the spore bank was noticeably smaller though still probably dominated by the nearby species. By 10 m, the spore bank was reduced to a size similar to that found at sites entirely dependent on long-distance dispersal, and was no longer dominated by the nearest source. This is consistent with what is known of spore dispersal (Peck *et al.*, 1990).

Size and species composition vary with depth in the soil.

At many sites, the spore bank reduces in size with depth but the proportions of +T and −T gametophytes in the cultures remain approximately constant (Fig. 2). Viable spores frequently occur down to a depth of 30 cm and they have been found at depths of over 1 m. However, at some sites, very different spore bank profiles were observed. Two examples illustrate this.

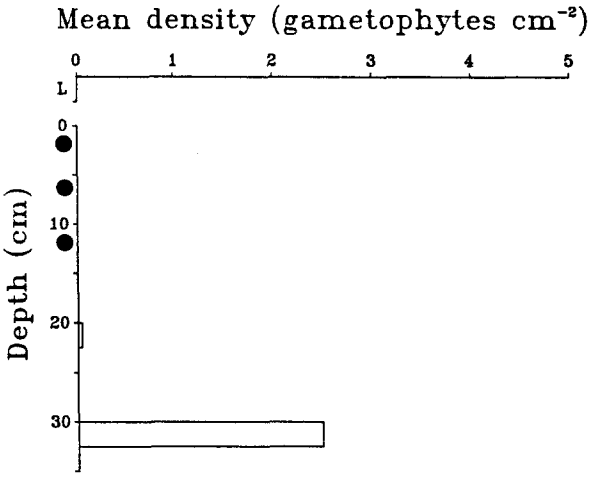

Figure 4. Spore bank profile for a sample site in an abandoned field near Pittsboro, Chatham County, North Carolina, USA (September 1987). The surface vegetation of grasses, compo-sites and a few brambles (*Rubus* sp.) with no pine seedlings indicated that cultivation had ceased between 3 and 10 years previously. No date could be obtained for the most recent forest clearance on the site. The nearest fertile ferns were a few scattered individuals of *Asplenium platyneuron* (L.) Oakes (+T) all more than 100 m away. There was no distinct litter layer. Samples were taken at 0–2.5 cm, 5–7.5 cm, 10–12.5 cm, 20–22.5 cm and 30–32.5 cm. Below 25 cm, the soil consisted of very compacted red clay. Viable moss diaspores occurred in every sample down to 20 cm and angiosperm seeds germinated in every sample down to 10 cm but both were absent at 30 cm. In contrast to this, the profile shows that viable fern spores were almost entirely restricted to the 30 cm sample. They gave rise in culture to very slow growing gametophytes, mainly in clumps. The gametophytes were too immature to accurately record the presence or absence of trichomes for all individuals but trichomes were present on some, and later examination revealed one with a young sporophyte resembling *Polystichum acrostichoides*.

(a) At a hillside site near Edinburgh, Scotland, in sterile bracken and more than 800 m from any fertile ferns, there was a marked change in species composition with depth within less than 20 cm (Fig. 3). This was consistent in all three sets of samples taken at that site. The change was largely due to an accumulation in the lower layers of spores of *Blechnum spicant* (L.) Roth, a species with very few gametophyte trichomes and scored as -T. No source of *Blechnum* spores was found in the vicinity, although a few small sterile plants were scattered over the same hillside. The nearest current source of the +T spores was over 800 m away. Apparently, therefore, all the spores were either old, deposited when the local flora was different, or of distant origin, or both. In the USA. *Blechnum spicant* shows high levels of interpopulation gene flow (Soltis and Soltis, 1990) indicating a capacity for long distance dispersal. Either way, there was no obvious explanation of the accumulation of *Blechnum* spores.

(b) In an abandoned field in North Carolina USA, there were no fern spores down to

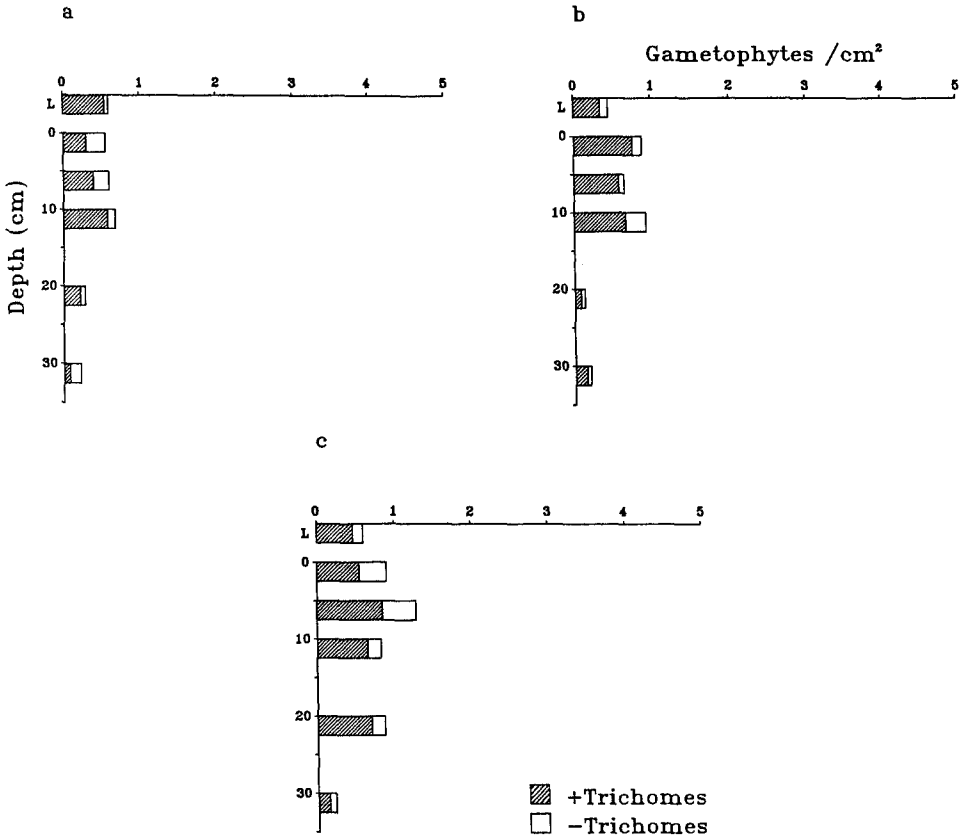

Figure 5. Spore bank profiles for sample sites in a consistently fertile population of bracken (*Pteridium aquilinum* subsp. *aquilinum*) (−T) in a hill pasture near Callander, Scotland. Profile a. was obtained just before (November 29, 1988) the spore release period and profiles b. just before (July 27, 1989) and c. just after (October 30, 1989) the next spore release. All three sample sites were within 1 m of each other. At 70 m or more distance there were fertile plants of *Asplenium trichomanes* subsp. *quadrivalens* D.E. Meyer emend. Lovis, *Athyrium filix-femina* (L.) Roth, (both −T) and *Dryopteris affinis* (Lowe) Fraser-Jenkins, *D. dilatata* (Hoffm.) A. Gray, *Oreopteris limbo-sperma* (All.) Holub. and *Polypodium interjectum* Shivas (all −T). Spores of *A. trichomanes* and *A. filix-femina* were found trapped on the bracken fronds surrounding the site. No bracken gametophytes were identified on the cultured samples and the spore bank was formed mainly if not entirely by long-distance dispersal (i.e. >70 m) from other species. The three profiles are similar, indicating that both recruitment and mortality are at a low frequency.

20 cm but a small accumulation occurred at 30 cm (Fig. 4). In the upper layers there were diaspores of weedy species of bryophytes but these did not occur at the levels where the fern spores were found. There were no fertile fern plants within 100 m and no spores had entered and remained in the upper layers during the 3–10 years it had been lying fallow. It is tempting to suggest that the bryophytes entered the upper layers of soil during the years of cultivation but the fern spores at depth survived from a previous forested period because they were below the level affected

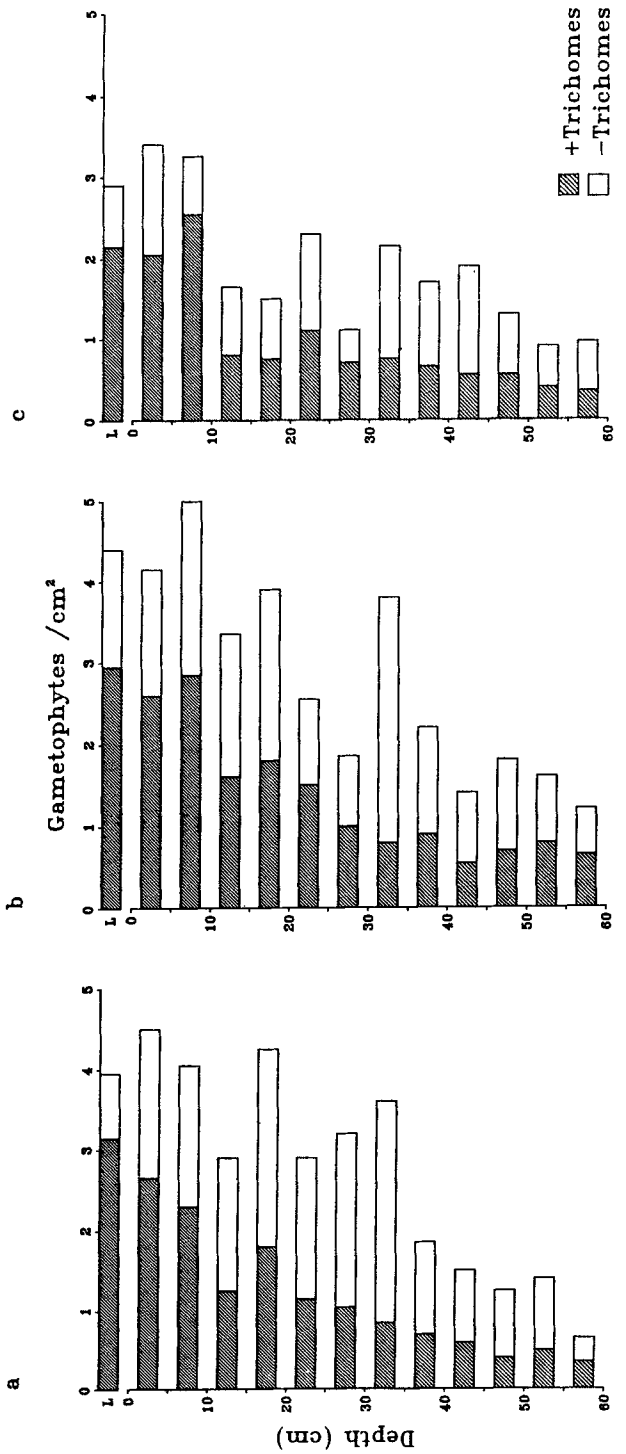

Figure 6. Spore bank profiles for a sample site in a small clearing in old deciduous woodland near Edinburgh, Scotland. The nearest fern sporophytes were *Athyrium filix-femina*, *Blechnum spicant* (both −T) and *Dryopteris* spp. (+T) about 1 m away. Profiles a. and b. were obtained on the same date (31.5.88) before spore release and at points 50 cm apart; c. was obtained 14 months later (31.7.89) midway between a. and b. Between the two sampling dates, the site was covered by a sheet of black polythene 1 m × 1 m pinned to the surface to exclude the spore rain. Profiles a. and b. are very similar, indicating that they accurately represent the viable fern spore populations at those points. They also indicate that substantial spore banks remain after at least 10 months in the soil. Profile c. reveals that a significant proportion (approximately 60%) of this spore bank remains after at least 2 years, if it can be assumed that the spores recorded did not move 50 cm laterally in the soil from outside the covered area. Almost all of the depletion occurs in the top 35 cm.

by the plough. Regular ploughing would remove any live fern spores initially present in the upper layers of soil by progressively bringing them to the surface where they would germinate during the wetter months and then be killed by the next ploughing, if not before.

Size and distribution of fern spore banks differ from those of seed banks and bryophyte diaspore banks

There were always more (up to 100× more) fern gametophytes than angiosperm seedlings in the spore bank cultures. Because only spores on the surface can germinate while most seeds at any depth will do so, the real difference in frequency is likely to be much greater than the results indicated. Angiosperm seeds were usually absent below 20 cm and in old forest sites. The distribution of bryophyte diaspores, including both meiotic spores and vegetative propagules, frequently differs from that of fern spores, as described above for the abandoned field site (Fig. 4). Whether this is due to differences in timing of deposition, or in longevity, or mobility within the soil, is not known.

Spores remain viable for at least a year

Samples taken at different times of year from a hillside in Perthshire, Scotland, all reveal spore banks (Fig. 5) even though spore release in the local species is seasonal, as in all temperate ferns. Clearly, the spore bank survives at least a year. Moreover, the stability of the spore bank at a site such as this, depending as it does on long-distance dispersal, suggests that the spores are long-lived, with a low level of mortality as well as recruitment. Other field experiments revealed that spore banks survive for more than two years (Fig. 6). Spores in dry storage in the laboratory or herbarium can remain viable for up to 70 y in some species (Lloyd and Klekowskl, 1970). Spores in the soil are likely to be hydrated but it is now known that hydrated spores, even the chlorophyllous ones, can remain viable longer than dry ones (Clymo and Duckett, 1986: Lindsay *et al.*, 1992) so it seems likely that spores of at least some species have the potential, other factors such as 'sporivores' permitting, to survive for many years in the soil.

Spore banks survive forest and heath fires

Samples taken from sites recently burnt to control forest undergrowth or to stimulate heather regeneration showed typical fern spore banks even in the upper layers of soil.

The implications of these observations for the understanding of fern reproductive biology are considerable and it is already clear that the life cycle of a fern species in the wild cannot be adequately described without reference to the spore bank. It is also now possible to assess these natural spore banks as a resource for conservation purposes. Seed banks have been considered in relation to conservation (Maunder, 1992) and *Senecio palustris*, presumed extinct in Britain, and *Iliamna corei*, endangered in the USA, have been retrieved from seed banks (Walters, 1974; Thompson, 1988). Fern spore banks differ from seed banks in several ways. Almost all ferns are perennial, whereas in angiosperms, seed banks are particularly associated with annual species. Fern spores are produced in extremely large numbers and can be more widely distributed than most seeds, so spore banks are more widespread and contain more individuals than seed banks. Recruitment to temperate fern spore banks is more seasonal than for seed banks because spore release in most ferns occurs within the same late summer/early autumn

period whereas some angiosperms shed seed much earlier in the year. Fern spores are much smaller than almost all seeds and consequently, movement into and within the soil is likely to be less restricted, which probably explains why fern spores have been found at greater depth than seeds at the same site. The imbibed spores of most fern species require only light at a suitable temperature to initiate germination; the seeds of some angiosperms have alternative or additional requirements because of other forms of inherent dormancy which affect the germination response. Despite these differences, spore banks also have a considerable conservation potential,

Natural spore banks as a resource for *ex situ* conservation

It is first necessary to determine whether endangered fern species can form spore banks. Less than 60 fern species have so far been positively identified in spore banks but this is in part due to the difficulties of identifying species at the gametophyte stage. Although none of those listed is endangered worldwide, there are indications that many more, though perhaps not all, fern species form spore banks. It seems reasonable to expect that at least some of the rare and endangered species in the UK and abroad do so. In Britain, several species have been reduced to a few populations, some with less than ten individuals. These include the species individually protected by law (*Cystopteris dickieana* Sim, *Ophioglossum lusitanicum* L., *Trichomanes speciosum* Willd., *Woodsia alpina* (Bolton) S.F. Gray and *W. ilvensis* (L.) R. Br.) and other species have been identified as threatened and in need of conservation, including *Asplenium septentrionale* (L.) Hoffm., *Dryopteris cristata* (L.) A. Gray, *Gymnocarpium robertianum* (Hoffm.) Newman and *Thelypteris palustris* Schott (Page, 1982; Whitten, 1990; Jermy and Camus, 1991).

That being so, it is appropriate to consider the likely advantages and disadvantages of using natural spore banks for *ex situ* conservation of those ferns which form them. The potential advantages are considerable. Soil spore banks are available throughout the year and can be collected with no disturbance to growing plants and minimal interference with natural regeneration from spores. Soil samples containing spore banks have been successfully stored for two years in a refrigerator, although it has not been confirmed that the gametophyte population that develops when these are subsequently cultured is identical to that from fresh samples; differential mortality rates, after-ripening or induced dormancy (Hamilton, 1988), for example, could cause changes in species composition. Spore bank samples are easily cultured and sporophytes produced on them can be reared to maturity.

In addition, spore banks might provide access to genotypes no longer present in living sporophyte populations that have been drastically reduced in size in recent years. Reintroduction of sporophytes raised from these spore banks would increase the genetic heterogeneity of the population which, in an outcrossing species, would in turn create more heterozygosity and perhaps thus greater vigour and ability to adapt in subsequent generations. It may be, as proposed for seed banks (Levin, 1990) despite the repair processes that seem to take place in imbibed seed (Villiers, 1975; Villiers and Edgcumbe, 1975), that spore banks accumulate novel genetic variation by mutation, some of which might even be potentially adaptive. There is however no evidence to test this hypothesis as it is rarely possible to even tentatively date the spores in a spore bank.

Another possibility is the retrieval from buried spores of sporophyte populations

entirely lost due to recent seral change or surface catastrophe such as drought, grazing, fire or cultivation. Dormant spores might even be more resistant than rooted plants to changes within the soil, such as waterlogging, drainage, pollution or pH shifts. Where the environmental changes are man-made and can be reversed by appropriate habitat management, re-introduction of native genotypes retrieved from spore banks might result in the successful restoration of the population. For example, when British pond and river margins and other wetland sites are taken out of cultivation as a consequence of the new agricultural 'set-aside' policy, it might be possible to restore the ferns native to the sites. When the lost population is also the last population of a critically endangered species, this restoration is effectively resurrection from worldwide extinction. This might be successful when the species is rare as a result of isolation or recent active evolution and its loss is due to human interference, but when it is an ancient species at the end of a long period of natural decline, the outcome is less certain.

The main disadvantages of spore banks as an *ex situ* conservation resource are that the samples are more bulky to store than newly released spores and that there are usually other species present in addition to the one selected for conservation, though this can be an advantage when the objective is to conserve a natural assemblage of species. Unsterilized soil samples are also subject in some countries to import/export restrictions which could complicate international transport.

Thus, although there might be some endangered species that do not form spore banks, for the others the potential advantages of using spore banks for conservation appear to considerably outweigh the disadvantages. Therefore, I have begun to look for confirmation that some of the expectations can be realized. Two examples are summarized here.

(i) Several plants of *Asplenium septentrionale*, together with 5 other species, have been raised from cultured samples of soil from a population of this species in Edinburgh. The samples were taken, just before annual sporing commenced, from the surface of the soil near growing plants. *Asplenium septentrionale* is scarce in Britain where its restricted habitat range limits it to a few widely scattered localities (Jermy *et al.*, 1978; Page, 1982). Although the site sampled is one of the classic locations for the species, where the population still exists 200 years after it was recorded by Bolton (1790), it has been greatly reduced in Britain over the last hundred years due to over-collecting and shading (Page, 1982) and was lost from almost half its recorded sites within 40 years after 1930 (Jermy *et al.*, 1978). Some of the remaining populations are very small and threatened with extinction if conservation measures are not taken. The demonstration that spore banks exist at sites of living populations creates new possibilities for restricting the futher decline of this vulnerable species. (Note added in proof: Subsequent investigations (Dyer and Lindsay, unpubl.) have revealed that *Cystopteris dickieana, Woodsia alpina, Gymnocarpium robertianum* and *Thelypteris palustris* also form persistent spore banks.)

(ii) In a more speculative investigation, samples were taken from a fen at Loch Libo, Renfrewshire, Scotland, which until approximately 40 years ago contained the last known Scottish population of *Dryopteris cristata* (L.) A. Gray. These samples have yielded many gametophytes in the organic soil down to about 10 cm and some in the mineral soil at 25–30 cm but none in the intervening sample at 20 cm. Mosses only grew on the organic soil down to about 10 cm and angiosperm seedlings were absent below 20 cm, so only fern gametophytes grew on the samples from 25 and 30 cm. Some of the fern gametophytes developing on the surface samples and a few

of those on the 25 cm sample have trichomes, a characteristic of *D. cristata* (L.) but also of most British species, including *D. dilatata* which is common in the surface vegetation surrounding the fen. The cause of the interrupted spore bank profile and the history of the spores isolated in the deepest samples is not known. While the chances of retrieving gametophytes from 40 year old spores of the lost species must be slight, these observations suggest that it might not be totally impossible.

Natural spore banks as a resource for *in situ* conservation

For up to 300 million years, soil spore banks have been evolving as a means of protecting populations in the wild against catastrophic elimination and genetic erosion. Accordingly, while the obvious conservation potential for natural spore banks is through the *ex situ* approach, another interesting possibility is that they could be used for *in situ* restoration, that is the retrieval of lost populations or even species at the original site by creating the circumstances for which spore banks are pre-adapted. If artificial disturbance of the soil to bring spores to the surface was followed by suitable growing conditions, spontaneous regeneration might follow. This might require the initial creation of local microhabitats to provide the shade, moisture, substrate stability and protection from competition necessary for the production of gametophytes and establishment of young sporophytes. When the cause of the species' decline is anthropogenic, overall habitat restoration at the site might also be required subsequently to maintain the conditions necessary for the mature sporophyte to succeed.

The way forward

Most ferns, even epiphytic and epipetric species, grow and shed spores into the soil or the similar substrates trapped in rock and tree crevices. It now appears that many, perhaps most, species form long-lived spore banks in soil. Therefore, the conservation potential for natural soil spore banks, summarized in Fig. 7, is considerable. However, if that potential is to be realized, there must be further research into the biology of spore banks, appropriate monitoring of remaining populations of endangered species, and the necessary taxonomic and horticultural support.

Research is needed into the distribution, composition and longevity of spore banks, the control of spore germination, the maintenance of spore viability under different storage conditions, and the *in situ* restoration techniques involving spore banks. This would be of particular interest for tropical species which include the majority of ferns. Monitoring of threatened populations is necessary to ensure that emergency rescue operations can be mobilised soon after, if not before, the population is lost. It will also be necessary after re-introduction or restoration to assess the success of the measures taken. Taxonomic supervision is necessary to direct and co-ordinate the collection of samples, to provide authoritative identification and to ensure the maintenance of a collection of voucher specimens. Horticultural support and laboratory facilities are needed to culture the soil samples, to raise and maintain the sporophyte collection and to service the soil sample storage facility. Over and above this, staff will be required to direct these conservation projects, and link them to other activities within the international Botanic Garden and conservation community, if these support activities are to be channeled into an effective programme.

Botanic Gardens are the institutions best placed to provide the required supervision,

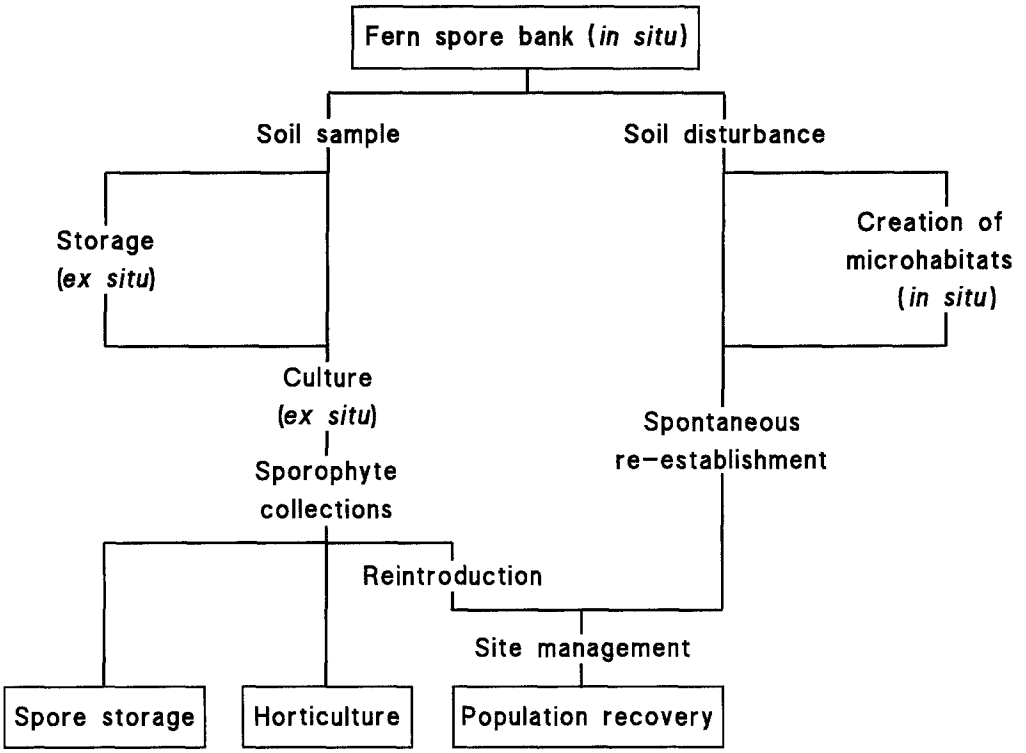

Figure 7. The role of soil spore banks in fern conservation.

services and facilities. RBGE is already meeting some of these requirements within its Pteridophyte Conservation Programme, alongside development of an *ex situ* spore storage facility and sporophyte collections. However, many of the threatened ferns are in tropical forest enclaves or islands and we need to cooperate with other appropriately situated gardens in order to extend this approach to tropical species. We would welcome contact with any organization interested in such cooperation.

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