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Spore bank, dark germination and gender determination in *Athyrium* and *Dryopteris*. Results and implications for population biology of Pteridophyta

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Abstract

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There exists a remarkable spore bank in the soil, mainly within sporophyte populations. The majority of the viable spores are found in the first 10 to 15 cm but they reach a depth of at least 65 cm. Erosion, turning over of soil layers, and disturbance may bring spores again to the soil surface. As a consequence of the spore bank, spores seem to be omnipresent throughout the season and will germinate whenever the conditions are favourable. The spore bank plays an important role also in relation to pheromone influence, gender determination and breeding system. Pheromones (antheridiogens) produced by female prothalli induce formation of antheridia and dark germination. At safe sites in the microhabitat where prothalli develop into females spores in the soil can be activated. Dark-germinated spores become male and may guarantee an effective and swift fertilization (outbreeding). It is likely that the number of spermatozoids represent a limiting factor for fertilization and that antheridiogens are means that enable the formation of sufficient male gametes.

Key words: Pteridophyta, *Athyrium, Dryopteris,* spore bank, dark germination, gender determination, gametophyte ecology.

Introduction

When studying the life cycle of homosporous ferns one has to deal with two independently growing generations, the gametophyte and the sporophyte. These two generations are characterized by striking morphological (organisational), anatomical and ecological differences. In studies of the population structure of a fern taxon the limits set by the adaptive abilities of both generations have to be taken into consideration. In *Athyrium filix-femina* (Schneller 1979), and certainly in most other ferns, the gametophytes show pioneer traits. In terms of the K- and r-models (MacArthur and Wilson 1967) gametophytes are to be regarded as r-strategists. The pioneer habitat, however, may consist of small disturbed patches within an established plant com-

munity such as woodland, or of large disturbed areas due to catastrophic events (Lloyd 1974a, 1974b), or of areas influenced by mean. In *A. filix-femina* and other wood inhabiting fern species, the gametophytes are found on small patches of open, bare soil, or on pieces of decaying wood or tree trunks. The colonization of such microhabitats is essential for the recruitment of new sporophytes into the population.

In many higher plants it is well known that seed dormancy and the size of the seed bank are of eminent importance for colonization (Harper 1977, Roberts 1981), mainly in pioneer plants such as weeds. Up to now we know little about spore banks in ferns. The presence of viable spores in soil was, as far as can be learned from the literature, reported for the first time by Wee (1974) who studied ferns and weeds in pineapple fields of varied ages in Malaysia. Strickler & Edgerton (1976) recorded spores of *Cystopteris fragilis* in soils of coniferous forests in Oregon. During & ter Horst (1983) reported fern spores as occurring in soils of Dutch chalk grasslands and recently Leck & Simpson (1987) showed that fern spores occur in soil of tidal wetlands of the Delaware River. However, all these studies were made in areas close to or a few kilometers from fern populations and not within their confines. In the present study the spore bank of soil samples taken within or close to fern populations is investigated. The biological and ecological implications of spore banks and a hypothetical relationship between spore banks and dark germination and gametophyte establishment are discussed.

Materials and methods

Distribution of spores in the soil was investigated by examination of four sampled sites within forests. The localities sampled were: Kanton St. Gallen (Switzerland), Ricken, Egg (one collection); Kanton Zürich (Switzerland), Horgen, Horgenerberg (two collections), and Herrliberg, Chüelenmorgen 1 (four collections) and Chüelenmorgen 2 (one collection); two sites, Horgenerberg and Chüelenmorgen 1, within fern sporophyte populations and one (Chüelenmorgen 2) in a sporophyte free habitat (about 50 m from nearest sporophyte population). The samples were taken between end of October and December 1987, which was at least a month after the spores were ripe. Holes were excavated to a depth of 65 cm. Core samples were collected using an aluminium reed of 2 cm inner diameter. The reed was placed in the hole and pushed horizontally into the soil at different depths down to 65 cm. For each chosen depth the two ends of the core sample were cut away and only the middle part was used to avoid potential contamination. The samples were put into clean polythene bags, brought to the laboratory, and were placed in polystyrol boxes ($4 \times 4 \times 2.5$ cm with cover). Sterile water was added if necessary and the boxes were stored at room temperature near a N-exposed window, in order to allow development of prothalli. After 3–5 weeks the presence of prothalli was determined with a dissecting microscope. The number of the prothalli (= germinated spores) counted was then related to the examined surface area (Fig. 1). No attempt was made to relate the number of viable spores to the soil volume.

From natural populations at Horgenerberg (Kanton Zürich, Switzerland) and Mt. Holyoke (Hampshire Co. Massachusetts, U.S.A.) small patches of soil with gametophytes were collected, their position on the soil surface was mapped, and the gender of the gametophytes was analyzed in the laboratory. Prothalli developing in the laboratory on a soil sample from Chüelenmorgen 1-1 were also mapped and the sex was studied (Fig. 3c).

For studying dark germination or gender development, either female prothalli or the substrate of established prothalli populations were used as an antheridiogen source. For germination experiments in the laboratory spores from the following plants were used: *A. filix-femina* (L.) Roth Nr. A-1 Horgenerberg, Horgen (Switzerland); *A. filix-femina* spp. *angustum* (Willd.) Liew Nr. Sch-475 and Sch-574 Amherst, Franklin Co. Massachusetts (U.S.A.); *A. filix-femina* Nr. Sch-1013, Lookoutpass, Shoshone Co. Idaho (U.S.A.); *Dryopteris filix-mas* (L.) Schott Nr. F-54 Oensingen,

Roggenflueh, Kt. Solothurn; Nr. F-56 Trin, near Crestasee, Kt. Graubünden; Nr. Sch-242, cultivated in private garden Küsnacht, Kt. Zürich (all Switzerland). *Dryopteris affinis* (Lowe) Fraser-Jenkins Nr. 16 and Nr. Sch-199 Horgenerberg, Kt. Zürich (Switzerland).

In an experiment on dark germination of *A. filix-femina* ssp. *angustum* a mixture of spores (Sch-574) and soil was enclosed in a small container covered by small-meshed Nyboldt net (width of mesh ca. 20 µ). The container was placed 1 cm deep in the soil substrate of a prothalli culture (Sch-475) and completely covered.

To investigate the viability of dark germinated protonema an experiment was started in October 17, 1984. Spores were sown in a fresh medium containing a few female prothalli of the same individual and kept in the dark up to March 22, 1985; then they were exposed to daylight.

In an earlier publication (Schneller 1979) the germination of *A. filix-femina* Nr. A-73 (Ricken, Switzerland) and Nr. A-2 (Horgenerberg, Horgen, Switzerland) was studied. Some of the results are incorporated into the present publication.

Results

Spore bank

It is possible to recognize prothalli of *A. filix-femina* in nature. In the sites used for this study it is the only fern species with prothalli without glandular cells (hairs). In the areas studied, *A. filix-femina* and species of *Dryopteris* are the dominant ferns. Within fern populations viables spores exist in the soil down to 65 cm (and possibly deeper). The relative density, however, decreases from 5 cm down to 65 cm. In Fig. 1a the distribution of the number of viable spores per 5 cm^2 is given for collection site Chüelenmorgen 1-1. The number of viable spores decreases significantly when soil from an area without ferns is examined (Fig. 1b). Fig. 1c shows the mean number of spores per 5 cm^2 calculated from all collections made within sporophyte populations. Note the relatively high number at depth 50 cm which resulted from finding a whole sporangium containing many viable spores. In all three examples (Fig. 1a, b, c) the lower frequency of *Dryopteris* is obvious. Three different *Dryopteris* species occur in the sample sites, and it is not possible to separate prothalli of different *Dryopteris* species by morphological characters; hence the frequency of viable spores is given for the genus (Fig. 1a, b, c).

Germination experiments

In an earlier publication on *A. filix-femina* (Schneller 1979) it was shown from laboratory experiments that soil from gametophyte populations and/or female prothalli induce antheridia formation and dark germination. Similar experiments with *D. filix-mas* and *D. affinis* showed a comparable effect (Schneller 1981). It is evident that in *A. filix-femina* and *D. filix-mas* a very similar or identical antheridiogen (pheromone) occurs because antheridia and dark germination can be induced in *Athyrium* by *Dryopteris* and vice versa. There is, however, some evidence for small differences (concentration?, chemistry?) because *Athyrium* responds differently in the dark to the antheridiogen of *Dryopteris* compared to its own (Fig. 2a, d). Dark-germinated protonemata of *Athyrium* and *Dryopteris* normally form one, sometimes two, antheridia (Fig. 2). In the experiments performed these antheridia regularly form fully viable spermatozoids but usually fewer than in antheridia formed in the light.

It could be also shown that in *A. filix-femina* (Plant Nr. Sch-1013) protonemata generated in the dark (due to antheridiogen influence) remain viable for a considerable

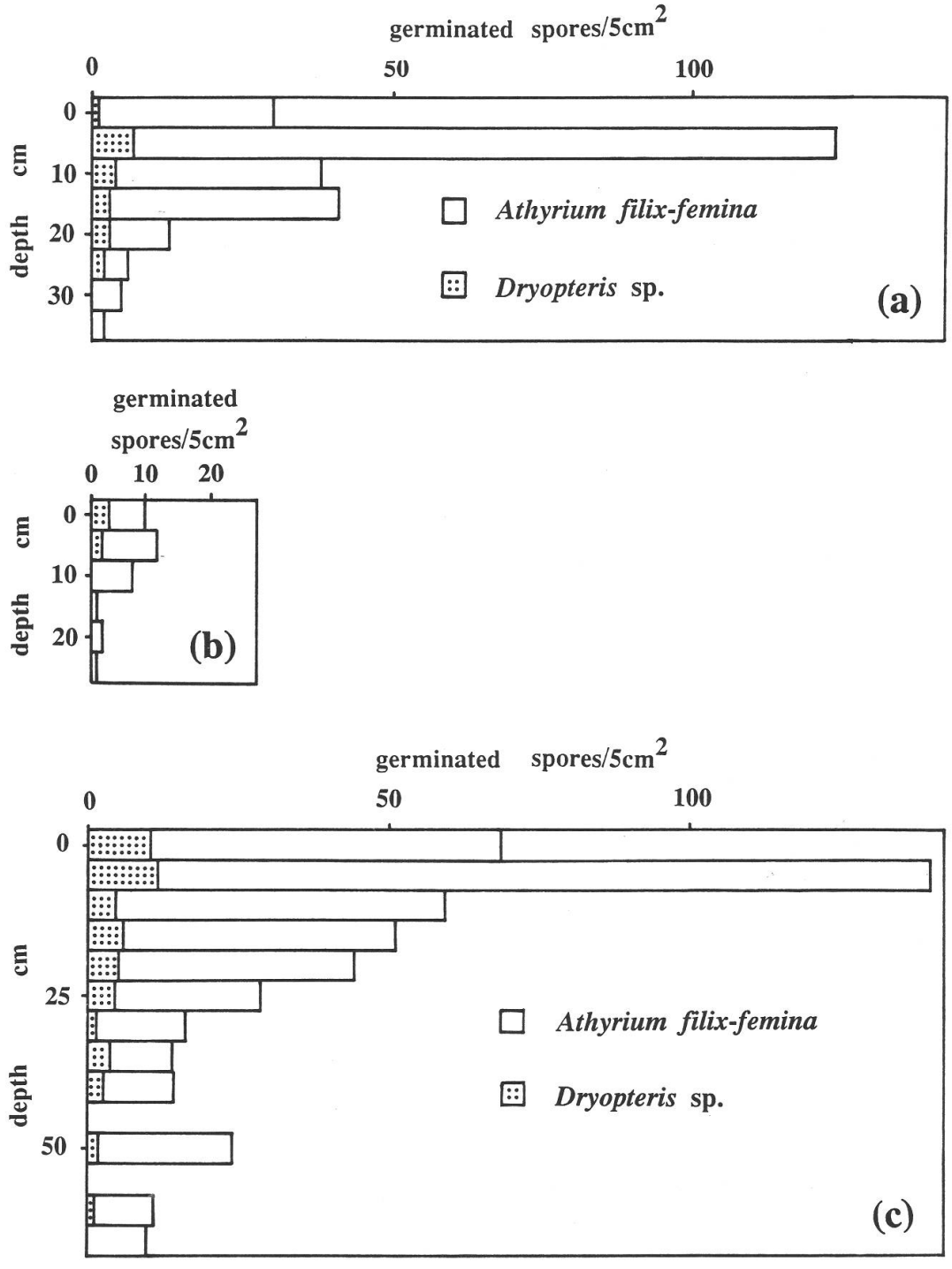


Fig. 1. Distribution of viable spores found in soil collected from different localities within forests. a). Spore bank of one locality at sampled site Chüelenmorgen 1-1; soil collected on October 3, 1987, and analyzed on November 10, 1987. b). Spore bank of site Chüelenmorgen 2, 50 m from nearest sporophyte population; soil collected on December 30, 1987, and analyzed on January 18, 1988. c). Spore bank of samples taken in all the seven studied populations; the mean value is shown. Note the relatively high number found at depth of 50 cm which was due to the occurrence of a whole sporangium containing many spores.

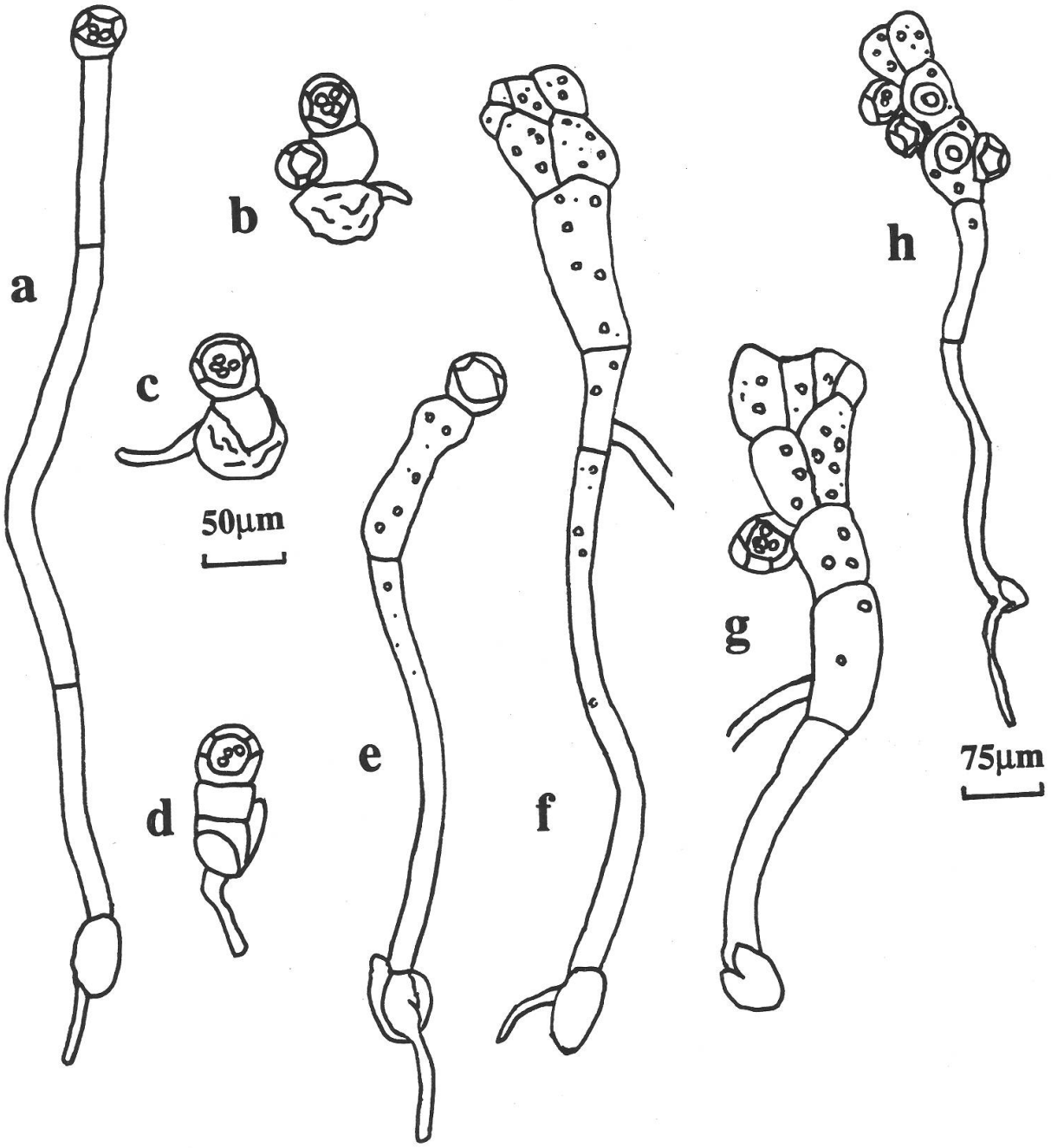
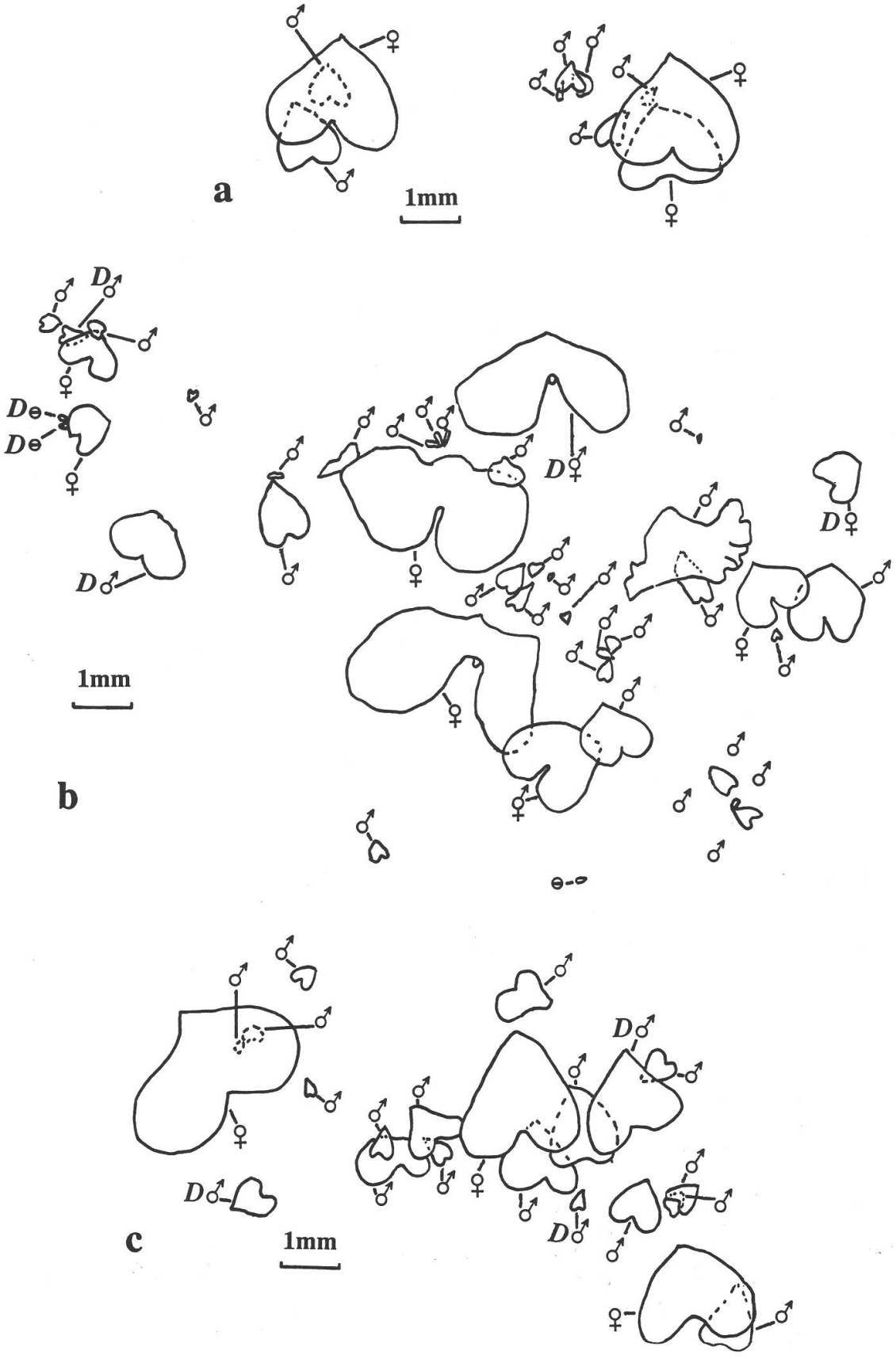


Fig. 2. Experiments and observations of germination. a). Protonema of *Athyrium filix-femina* (Sch-1013) germinated in dark under the influence of pheromone of the same species. One antheridium formed at the end of the protonema. b). Dark-germinated protonema of *Dryopteris filix-mas* (F-56) induced by pheromone of the same species. Note that the protonema is very short but two antheridia occur. c). Dark-germinated protonema of *Dryopteris affinis* (Sch-199) induced by *D. filix-mas* (F-54). d). Dark-germinated protonema of *A. filix-femina* (A-1) induced by pheromone of *D. filix-mas* (F-54). In contrast to a) the protonema remains short. e), f). Protonemata of *A. filix- femina* (Sch-1013) kept in dark from October 17, 1984 to March 22, 1985 and then exposed to daylight; developmental stages observed on March 29, 1985. Note the thicker chloroplast-containing cells that developed in light. g). Young prothallium of *A. filix-femina* collected in a natural population at Horgenerberg (autumn 1977) with thin first cell lacking chlorophyll, and with antheridium. h). Young prothallium of *Athyrium filix-femina* collected from culture from Chüelenmorgen 1-1, with the first cells as in g) and many (pheromone induced) antheridia.


a
1mm
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1mm
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span of time. After 5 months in the dark they were still viable and when exposed to daylight after a few days developed green cells (Fig. 2) and grew into regular heart-shaped prothalli.

When the buried container with a spore and soil mixture (see materials and methods) was checked after three weeks, many dark germinated protonemata with antheridia (like Fig. 2 a) could be observed.

Prothalli populations and gender

The distribution and sex of prothalli collected from natural populations and prothalli growing on soil samples collected in nature and grown in the laboratory are shown in Fig. 3. In all prothalli populations studied (small) males and (well developed) females predominate and only occasionally hermaphrodites occur. In all populations, prothalli with the first-formed cells similar to those found on protonemata growing in the dark (Fig. 2 e, f, g, h) could be found.

Discussion

In a comparison of the distribution of spore banks with that of seed banks (Harper 1977, Roberts 1981) it should be noted that fern spores seem to be found naturally at greater depths than most seeds. In some small-seeded genera, however, such as *Striga,* seeds are found to a depth of 150 cm (Harper 1977). In our experiments samples were taken not deeper than 65 cm and it is quite likely that some spores may be found at greater depth. Both *Striga* seeds and fern spores are small, and there seems to be a correlation between depth that can be reached and diaspore size. The question is how these diaspores became buried. A very plausible explanation is that it is due to the activity of earthworms which may reach much more than 1 m of depth in some types of soil (Darwin 1881, Müller 1965). The diaspores may be washed into these worm holes or they may be carried actively into the soil. It is well known that earthworms draw litter parts into the deeper zones of the soil. Another explanation is that spores may pass through the gut of the earthworms without being damaged. If this is true, spores might be brought back to the surface in deposits of excreta.

The soil samples of earlier investigations were collected only to a depth of 15 cm (Wee 1974, During & ter Horst 1983, Leck & Simpson 1987), and all were taken either away from sporophyte populations or in areas where only a few ferns occurred (Leck & Simpson 1987). The results, however, show that spore rain and short- or even long-range spore dispersal occur. This long-range dispersal and the occurrence of viable spores within the soil seem to be very important for the establishment of new populations and the colonization of new sites.

Fig. 3. Gender distribution of prothalli populations. a). Population from Mt. Holyoke (Mass.), collected in August 1983; sex distribution of unidentified gametophytes. b). Population from Horgenerberg (collected autumn 1977) with prothalli of *A. filix-femina* (no marking) and of *Dryopteris* sp. (marked with D). o = sterile gametophyte. c). Population on a soil sample taken from Chüelenmorgen 1-1 (October 1987) and grown in the laboratory; *A. filix-femina* (no marking) and *Dryopteris* (marked with D). Note that in all the populations (a–c) the majority of the prothalli are either male and small or female and taller. Hermaphrodites are found only occasionally.

There is a striking difference between the numbers of buried spores reported from samples taken within and samples taken outside sporophyte populations (Fig. 1, Wee 1974, Leck & Simpson 1987). This is in good agreement with the reported results from studies of spore rain and spore dispersal within and outside populations (Schneller 1975, Conant 1978). The vast majority of all spores are deposited in a circle of 5 to 10 m around an individual spore source. The leptokurtic distribution allows dispersal over longer distances so that spores reach the soil far beyond sporophyte populations. The spore rain, however, is restricted to late summer and the beginning of autumn (July–August). The portion of *Dryopteris* sp. spores in the samples (Fig. 1) is significantly smaller than that of *A. filix-femina.* This seems to be mainly due to the lower frequency of *Dryopteris* sp. compared to *A. filix-femina* in the areas selected for this study. Phenological observations indicate that in addition there may be a higher spore productivity in *A. filix-femina:* In *A. filix-femina* fertile leaves are formed nearly throughout the whole spore-producing season (from June to September) whereas in *Dryopteris* the number of fertile leaves is determined early and fixed for one season. The fertile leaves appear in May and no additional ones are produced.

Little is known about dormancy and life-span of spores within the soil. Observations from my own collections show them to remain viable in the forest soil for at least one year. Spores of *D. filix-mas* and *A. filix-femina* kept in herbaria or stored dry at room temperature remain viable for at least 2½–3 years (Schneller 1975, Schneller 1979). Most probably spores in the soil may remain viable for many years. Further experiments should be made to learn more about dormancy and life-span of fern spores under natural conditions.

The consequence of this impressive spore bank, mainly within sporophyte populations, is that during the whole season spores are omnipresent whatever happens to the surface of the soil (erosion, disturbance by soil organisms, earthworms etc.) and spores germinate whenever the conditions are favourable. Even outside fern populations (Fig. 1, Wee 1974, During & ter Horst 1983) a considerable number of spores per unit area can be observed. In contrast to the opinion of Grime (1985) that ferns have no capacity to regenerate from spore banks, spore banks probably are of great importance and are necessary for colonization by gametophytes, quite similar to pioneer strategies of r-plants (MacArthur and Wilson 1967). The pioneer sites in this study are small, relatively unstable patches within the forest community.

The spore represents the first stage of sexual generation. In ferns (and other cryptogams) it represents also the diaspore. One cannot discuss spore bank without also considering gametophyte development, gender determination and establishment of new sporophyte populations. It was shown for many ferns, including *Athyrium* and *Dryopteris,* that pheromones (antheridiogens) are produced which are important in regulating sexuality and germination (Schneller 1979, 1981). In *A. filix-femina* and in the *D. filix-mas* group (and other ferns as well, see Näf 1979) the pheromones have also a secondary effect; they induce dark germination which otherwise does not take place. From this study it can be seen that, although *A. filix-femina* and *D. filix-mas* seem to have a very similar pheromone, there exist intrageneric differences (Fig. 2a, b, d) which may affect competition. It appears important that dark-germinated spores are able to produce (in the dark) at least one antheridium and mobile, fully viable spermatozoids. From Fig. 1 it is evident that a vast amount of spores are buried in the soil and remain dormant. In favourable, relatively stable and undisturbed sites (mainly small patches of bare soil etc. on the forest floor) prothalli develop and reach the stage where they start to produce pheromones (antheridiogens). In *Pteridium aquilinum* (Näf 1979) and in

A. filix-femina (Schneller 1979) this stage is just before the formation of the archegonia. The occurrence of prothalli with long thin first cells (Fig. 2g, h), the distribution of sexes, and the gender-specific size classes suggest that antheridiogen functions in nature (Fig. 3a, b, c; see also Schneller 1979).

It must be emphasized that the highest frequency of spores in our observations (Fig. 1) is not found at the surface but at about 5 cm depth. The surface layer in a forest contains mainly litter (leaves, needles, part of dead branches, etc.). This is relatively coarse material and, when rain falls, the spores are washed out and carried deeper into the soil. There the spores are filtered by the fine-particled layers of the soil. Considering the observations and experiments, it is likely that when pheromone is produced and reaches these spores, dark germination is induced. Spores close to the surface (possibly up to 1 mm) may, after induction of germination, reach the surface and the light, be further influenced by pheromone, and develop into normal male gametophytes. Results in this study (Fig. 2e, f, g, h) support this hypothesis. Under pheromone influence spores of deeper layers develop into dwarf males (thin long or short protonemata with one or sometimes two antheridia (Fig. 2a, b)). In these experiments it was shown that the pheromone reaches spores down to at least 1 cm. Schraudolf (pers. comm.) observed that the pheromone produced by one female prothallus of *Anemia phyllitidis* reached spore targets as far as 15 cm below the source. Experiments in the laboratory (Schneller unpubl.) demonstrated that spermatozoids may swim for distances of about 10 cm. It is likely that they also can pass through porous soil.

At favourable sites where females develop, many spores resting in the soil are activated and are "forced" to develop antheridia. One could then speak of a three-dimensional "male field" around pheromone producing (mature or almost mature female) prothalli which guarantees the production of enough male gametes to assure effective and swift fertilization. It is likely that in terms of chances for fertilization spermatozoids are a limiting factor.

Surprisingly even dark germinated protonema seem to be very resistant and durable because after 5 months in the dark they developed into normal green prothalli when brought to light (Fig. 2f). When they accidentally reach the surface they remain alive and continue to develop.

The occurrence of a very effective spore bank affects our ideas about the ecology of the regenerative phase. In contrast to the ideas of Grime (1985) I believe spore banks to be of great importance. Grime (1985) proposed that two regenerative strategies are strongly characteristic of fern gametophytes: regeneration through widely dispersed spores and regeneration involving persistent juveniles. A third strategy must be added: regeneration involving persistent spore banks. In comparison to the reproduction by seeds the disadvantages of an independently growing gametophyte generation are to some degree balanced by the production of a vast number of spores which are omnipresent in fern populations. In addition, the regulation of germination and gender determination, and the mobilization of hidden spores through pheromones promote outbreeding and are further means that allow competition with the "more successful" seed plants.

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