The Biology of *Athyrium distentifolium* and *A. flexile* in Scotland

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Abstract

The Alpine Lady-fern, *Athyrium distentifolium* Tausch ex Opiz is found throughout the northern hemisphere. In Scotland, there is a smaller endemic form which was discovered in 1852 and has been named Newman's Lady-fern, *Athyrium flexile* (Newman) Druce. *A. flexile* is usually found with *A. distentifolium*. Although morphologically distinct, the taxonomic status of *A. flexile* has been the subject of continuing debate. This thesis aims to clarify the relationship between the two taxa.

Sites were located and characterised with descriptions of the vegetation and the montane environment. Two field sites were visited regularly throughout two growing seasons to monitor marked plants. This provided field observations to complement cultivation experiments. A morphometric analysis showed little overlap between the taxa for wild grown specimens. Comparisons between the spores and stomatal density showed small differences. The chromosome number was confirmed as being the same for both taxa.

Gametophytes and sporophytes of both taxa grown at low nutrient levels demonstrated that the smaller *A. flexile* matured faster and became fertile while *A. distentifolium,* which is usually larger and more vigorous, remained small and infertile. The *flexile* taxon may therefore be an ecotype which is specially adapted to stressful environments. *A. flexile* is only found in a limited number of sites in the Central Highlands of Scotland which have a low-nutrient substrate. It appears it can only survive in habitats where the competition from *A. distentifolium* is reduced. The most significant discovery from the cultivation of spores from individuals of *A. distentifolium* was that some plants, when self-fertilised, produced both the *flexile* and *distentifolium* type of sporophyte. *A. flexile* plants always bred true when self-fertilised. When gametophytes of *A. distentifolium* and *flexile* were given the opportunity to cross fertilise, some *A. flexile* gametophytes produced an *A. distentifolium* sporophyte. This suggests that *A. flexile* might be a homozygous recessive and does not justify species status.

Acknowledgements

In 1994, accompanied by Stuart Lindsay, I made the first of many treks into the great corrie at Ben Alder. We were looking for a small obscure fern, found only in a few sites in Scotland. If I was going to spend three years studying it, I wanted to be quite sure that I believed in its existence. This is the story of three years looking for the plant, growing it, and puzzling over a range of alternative ideas. Is it a species, a variety, an ecotype? Or is it, as the farmer at one field site said "Just a stunted wee thing that hasnae grown properly"?

Many noble and hardy friends have accompanied me into the hills. Grateful thanks to David Ellis, Stuart Lindsay, Jean and Andrew Gilchrist, Mary Clarkson, Margaret Abel, Muriel Clark, Martina and Graziano Rossi, Robin Walls, Clive Jermy, Barry Meatyard, Rosemary Smith, Ross Lindsay, Charlotte McHaffie, Lucy Haddow, Naomi Knights, Lynne Farrell, Gail Jackson, Sally Henderson, Geoffrey Harper, Susan Robertson, Alison Wilson, Sandra Stuart, Peter Hainsworth, Carole McLay, Jan Jeník, Colin Legg, Chris Sydes, Reg Mitchell, Steve Munyard (Jun.), Ted Munyard, Paul Ripley, and Bob, the Bed and Breakfast dog. Only the last of these thought it appropriate to sit on my ferns, and loudly refused to climb rocky slopes (or allow me to do so).

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CHAPTER ONE General Introduction

1.1 Introduction

In Scotland, there are two forms of the Alpine Lady-fern. There is *Athyrium distentifolium* Tausch ex Opiz (Figure 1a) and another endemic taxon that has been named *Athyrium flexile* (Newman) Druce (Figure 1b). These names will be used throughout most of the thesis. Since it was first found in 1852, there has been doubt over the taxonomic status of *A. flexile* and little was known of its ecology and distribution or abundance. It had not been established whether populations of *A. flexile* are expanding or rapidly declining. In The Fern Atlas (Jermy *et al*, 1978) there were five pre-1950 10-km squares with no recent records, and only five post-1950 records. With so few records *A. flexile* is listed in the UK Biodiversity Action Plan and the results of this investigation will provide recommendations to Scottish Natural Heritage. The thesis format is based around preparation for an account on *A. distentifolium* and *A. flexile* in the Biological Flora of the British Isles series (Elkington *et al*, 1975).

The aims of this study are to:

- clarify the relationship between *A. distentifolium* and *A. flexile*
- establish the distribution and abundance of *A. flexile*
- make comparisons between Scottish *A. distentifolium* and the same taxon elsewhere
- conduct cultivation experiments to establish that both taxa breed true
- conduct cultivation experiments to investigate the conditions under which both taxa grow most favourably
- investigate factors that might restrict the distribution of these taxa
- make recommendations on the status and conservation of *A. flexile*

Figure 1a *A. distentifolium* Tausch ex Opiz and **1b** *A. flexile* (Newman) Druce

This first chapter describes the discovery of *A. flexile*, with a description of both taxa. Chapter Two is a brief literature review concentrating on *A. distentifolium* with only a short section on *A. flexile*, reflecting the lack of available sources. Chapter Three is designed to define the physical characteristics of the environment in which *A. flexile* is found and to look for qualities in the field sites that are particularly distinctive for this taxon. There are detailed observations on the sites where *A. flexile* has been located during the study period, with notes on other locations that were visited. The vegetation is described and temperature was measured to illustrate the local climatic conditions. The fourth chapter is concerned with morphological comparisons between the two taxa, the object being to look for measurable differences and assess how consistent they are on a taxonomic basis. A morphometric analysis compared *A. flexile* with *A. distentifolium* from within Scotland and from elsewhere. This is followed by comparisons of the number of cells in the annulus, the spores, stomata, chromosomes and a survey for the presence of mycorrhizas. These topics also contribute to the Biological Flora account.

Chapter Five is a blend of experimental work on the life cycle contrasted with the annual cycle observed in the field on monitored plants. Detailed comparisons between *A. flexile* and *A. distentifolium* were devised to assess growth responses from spore germination through to the mature sporophyte. The objective was to define the specialised conditions *A. flexile* requires for successful propagation and growth and, if possible, contrast these with *A. distentifolium*.

The sixth chapter continues to explore some of the factors that might influence successful reproduction and connects field and experimental evidence to an isozyme study. This adds to the general delineation of *A. flexile*'s origin and distribution. The final chapter summarises the differences between *A. distentifolium* and *A. flexile* and suggests alternative interpretations of the available data.

1.2 The Background to these taxa

At the beginning of the nineteenth century, fern taxonomy was in a state of considerable uncertainty relating to the determination of species. Certain taxa now clearly recognised as species were thought to be varieties and others which appeared to be distinct species have since been reclassified. *Athyrium distentifolium,* the Alpine Lady-fern, (then called *Polypodium alpestre*), was recognised on the continent long before it had been found in Scotland. With increasing travel into montane areas *A. distentifolium* was first recognised in 1844 at Caenlochan (Newman, 1851) and frequently found thereafter. In the Summer of 1852 James Backhouse Snr. noted "A remarkable variety, with deflexed pinnae was met with in one place in Glen Prosen" (Backhouse, 1852). The specimens (Figure 1.2) were shown to Edward Newman who was immediately convinced that a new species had been discovered.

Newman had already decided that it was not appropriate to classify the Alpine Lady-fern as *Polypodium* and in 1851 had proposed the name *Pseudathyrium*. This recognised the similarity to *Athyrium* while acknowledging the virtual lack of an indusium. He named the new species *Pseudathyrium flexile* and published a detailed account of both *Pseudathyrium alpestre* and *P. flexile* in *The Phytologist* in May 1853. The two taxa were included in *A History of British Ferns* in 1854. His original descriptions were accurate and detailed:

> "*Pseudathyrium alpestre*. Habit rigid, frond lanceolate, sub-erect 2-3 feet long, bi-pinnate; pinnae ascending, distant near the base, elsewhere crowded, subacute; pinnules 25-35 on each side of mid-rib of pinna, wider at base, crowded, toothed; cluster of capsules 25 or more on each pinnule, crowded, finally confluent.
>
> *Pseudathyrium flexile.* Habit lax, flexile, frond strap-shaped, spreading horizontally, 8-18 inches long, bi-pinnate; pinnae distant throughout, horizontal or drooping, subobtuse, pinnules 7-10 on each side of mid-rib of pinna, narrower at base, distant, subobtuse, serrated; clusters of capsules 6-8 on each pinnule, distant, always separate, crescentic margin of attachment over sporangia at first."
> (Newman 1853)

Figure 1.2 First specimens of *A. flexile* found in Glen Prosen July 24th 1852

Newman did not indicate where the specimens he originally described have been deposited. Herbarium specimens from the Backhouse collection in the Royal Botanic Garden Edinburgh are labelled "First discovery in Glen Prosen" and have the appropriate date (Figure 1.2). It would seem reasonable to assume that these were the same specimens that Newman examined except they are named "*Polypodium flexile*" not *Pseudathyrium flexile* as Newman suggested. Only one small herbarium specimen in The Natural History Museum in London is from the correct locality, is credited to the appropriate collectors, and bears Newman's proposed name, but it is on a mixed sheet and undated.

His classification was not immediately accepted. The Backhouse family who, together with Thomas Westcombe originally found the taxon, seemed reluctant to use Newman's name *Pseudathyrium*. Their 1852 specimens labelled "*Polypodium flexile*" gave "Moore", or "Moore and Babington" as the authority. Thomas Moore did not recognise Newman's new species as anything other than a variety and in *The Nature Printed British Ferns* produced in 1859 he used the name *Polypodium alpestre* var. *flexile*. So although the Backhouses did not use Newman's name, they did not correctly use Moore's either. In 1855 on a further visit to Glen Prosen, more specimens were collected by the Backhouses and this time the herbarium sheets at the Royal Botanic Garden Edinburgh were labelled "*Pseudathyrium flexile*" although one label included a question mark. However the status of *Pseudathyrium flexile* remained unclear, for in 1859 Thomas Moore wrote: "It is certainly a very distinct variety, and very constant, probably a variety rather than a species, this moreover being the view adopted by its discoverer Mr Backhouse, who writes: 'Dissimilar as it is from *P. alpestre*, I shall continue doubtful of its specific difference if it does not turn up in other places'." (Moore, 1859).

Thereafter, all specimens were labelled either *Polypodium* or *Pseudathyrium* with *A. flexile* sometimes a variety, sometimes not. Different Floras adopted one status or another and repeated the same information through several editions. There is some confusion over who first used combinations of names and different authorities are

quoted, not always accurately. Moore referred to the generic name of *Athyrium* in 1859 but it was not in general use for some decades until 1886 when Boswell (or Boswell-Syme, or Syme) used Milde's 1867 classification of *Polypodium alpestre* as *Athyrium alpestre*. Boswell-Syme continued the debate around *A. flexile*'s status when he wrote: "I have great hesitation in separating this as a subspecies from *A. eu-alpestre*, because the character of the basal part of the frond being soriferous and not the apex is so unusual among Ferns, that it may be suspected to be an abnormal form or monstrosity, and as this I should have regarded it had Mr Backhouse's original station in Glen Prosen been the only one in which it occurred. But the Ben Alder specimens are similar, and in cultivation the plant becomes even more dissimilar from *A. eu-alpestre* than the wild specimens" (Boswell *et al*, 1886).

Druce, in *The Comital Flora of the British Isles* (1932) gave *A. flexile* species status, named it *Athyrium flexile* (Newman) Syme, crediting Boswell-Syme as the source of the first use of the revised name, although Boswell-Syme himself had expressed doubts and called it a subspecies. Page retained the species status and followed Clapham, Tutin and Warburg (1952) in giving Druce as the authority, now modified to *Athyrium flexile* (Newman) Druce (Page, 1997) Alternatively, the revision of *alpestre* to *distentifolium* by Tausch after Opiz (Steudel, 1841) gave the varietal name for Newman's Lady-fern as *Athyrium distentifolium* Tausch ex Opiz var. *flexile* (Newman) Jermy (Jermy *et al*, 1978). The debate has continued and the precise nature of the *flexile* taxon has remained unresolved.

3.1: Description

Athyrium distentifolium (Figure 1a) has bi-pinnate ovate-lanceolate yellow-green fronds from 20 to over 100 cm high arising in a shuttlecock from a central crown. The straight stipe is one fifth to one quarter the length of the blade with pale brown scales, usually broad, but occasionally narrow. The pinnae are widely spaced near the base of the frond, where they may be deflexed, and are crowded near the tip. The pinnae meet the rachis at an angle of 90° at the midpoint of the blade, or may be slightly ascending. The pinnules are broader at the base, tapering to a point (Figure

1.3). The sori are circular and have rudimentary indusia with irregular filaments when young, which are soon obscured as the sori grow. The sori may be concentrated in the upper part of the frond or extend towards the base. There is considerable variation in the number of sori depending on the size of the frond. Plants are frequently infertile. *A. distentifolium* is diploid, 2n = 80.

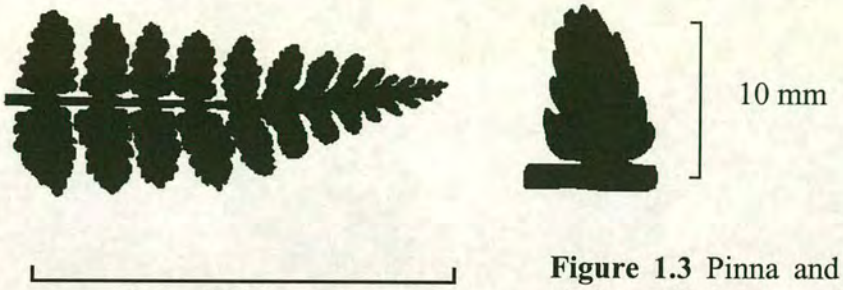


Figure 1.3 Pinna and pinnule of *A. distentifolium*

Athyrium flexile has bi-pinnate, narrow, blue-green fronds 10 to 40 cm long (Figure 1b) arising from a central crown. The fronds may be erect or sharply angled near the base of the rachis so as to lie nearly flat against the substrate. The stipe is short, one sixth to one eighth the length of the blade. It is often densely covered with broad, pale scales which can continue beyond the midpoint of the blade. The blade can be broadest near the base, or nearer the mid-point. The pinnae near the base are close together and often strongly deflexed at least half way up the frond. The uppermost pinnae are widely spaced. The pinnules taper towards the base (Figure 1.4). The sori are circular, although sometimes there are only a few sporangia. A rudimentary indusium is visible while the sori are still immature. The sori are concentrated around the base of the frond, extending towards the tip. The plants are usually fertile. *A. flexile* is diploid, 2n = 80.

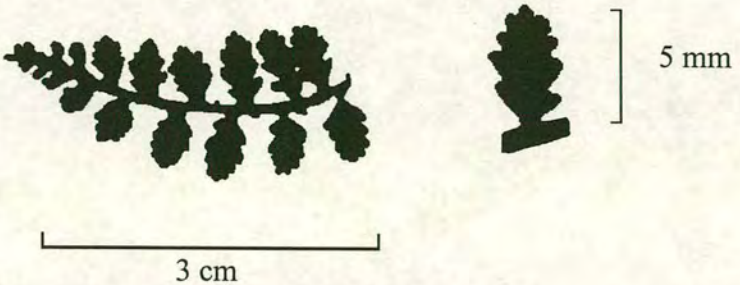


Figure 1.4 Pinna and pinnule of *A. flexile*

CHAPTER TWO Literature review

2.1: Introduction

This chapter is a review of the information that was mostly available before the study commenced. Most of the references in the literature are to *A. distentifolium* and these occupy a major part of the chapter. The section headings follow the format of the Biological Flora of the British Isles series. A brief section on *A. flexile* at the end lists the localities which have been recorded together with a few relevant references under the appropriate headings. Subsequent chapters develop the areas which are surveyed here, adding field and experimental evidence.

Athyrium distentifolium

2.2: Geographical and altitudinal distribution

Athyrium distentifolium is a circumpolar species. In the Atlas Florae Europaeae (Jalas and Suominen, 1972) *A. distentifolium* is shown in the north and east of Iceland, extensively distributed on the west coast of Scandinavia, in Scotland, (but not England or Wales), the mountains of Europe and eastwards into the former USSR. Il'in (1968) gave the distribution in the former USSR as in the subarctic zone, mountain woods near the timberline, in the alpine scrub zone and occasionally alpine meadows. This species is widely found in Lapland, the Northern Urals, the Caucasus, Western Siberia and the Far East. Sato (1982) studied *A. distentifolium* in north Japan.

A. distentifolium is found in North America and is called var. or subsp. *americanum*. It is described by Cody and Britten (1989) in arctic and alpine to subalpine habitats in the south of Greenland, Newfoundland and the Gaspé peninsula in Quebec. On the west coast it is found from south east Alaska through west and south British Columbia, Nevada and Colorado.

The altitude varies with latitude. Odland (1981) found *Athyrium distentifolium* in some parts of Norway as low as 300 m a.s.l, but he also described fern meadows in Western Norway between 650 and 800 m. Davis (1965) described the southern European *A. distentifolium* on rocky mountains from 2000 to 2700 m. Schneller and Rasbach (1984) give a range in Switzerland from 900 m to 2200 m.

Within Scotland, *A. distentifolium* is a scarce arctic-alpine fern (Rodwell, 1992), a member of late-lying snowbed communities found from 600 m to over 1000 m, present in the central and northern Highlands, usually on open, acidic screes. The subalpine woodland with which it can be associated elsewhere, is not present in this country.

2.3: Habitat

2.3.1: Snowbeds in Scotland

In Scottish sites, snow can begin to accumulate over the fern beds from October, and in exceptional seasons last until June or July. Overwinter snow provides a specialised habitat for plants which can tolerate long periods under the snow. As this cover is not altogether reliable, this further limits the range of plants which can grow in these areas. McVean (1958) described the *Cryptogramma crispa-Athyrium distentifolium* community as the best example of a type of vegetation which is completely dependent on winter snow protection. If there is a major reduction in the extent of the snow cover, dead plants would indicate areas that are usually covered, but which lacked the protection they normally received. Should any plants other than bryophytes be covered for more than one season, they too would eventually die. Snow in the Cairngorms reaches a maximum depth in March or early April diminishing thereafter. The depth of snowfall in late winter, influenced by the prevailing winds, determines how long the snow lies into the summer (McVean, 1963). There is usually no frost on the fern slopes during the short growing season (McVean, 1958).

Poore and McVean (1957) compared the continental climate, which has a high summer maximum temperature, low winter maximum and a rapid transition between, with the Scottish oceanic climate which has a flattened temperature curve with less extremes. This reduces the growing season. The oceanic climate is also more windy. Although there are thick accumulations of snow in the west of Scotland, spring rain rapidly melts this snow. In the east there is low rainfall and higher daytime temperatures but clear skies lower the night temperatures and the snow lasts longer (Poore and McVean, 1957). Early snowmelt increases the danger of frost for *A. distentifolium* which might have started into early growth, and this limits the westward distribution of *A. distentifolium*. It does not have the frost tolerance of the more widespread *Oreopteris limbosperma* and *Blechnum spicant* which are dependent on snow protection at higher altitudes in the east, but not in the west (Rodwell, 1992).

Precipitation on *Athyrium distentifolium* snowbed vegetation in the period 1941-1970 varied from 1600-3200 mm in the west to 1200-1600 mm in the east Grampians (Met. Office, 1979). During the same period in the Central and North-west Highlands (Met. Office, 1979) the January mean minimum temperature (screened at 1.25 m), was -5.0 °C (corrected by 0.5 °C for each 100 m to give approximate temperatures at altitudes of around 800 m), with a mean maximum of 1.0 °C (corrected by 0.7 °C for each 100 m to give approximate temperatures at altitudes of around 800 m). The July mean minimum for the same period, (Met. Office, 1979), was corrected to nearer 6.0 °C, with a mean maximum corrected to 12.4 °C. The mean number of days with snow lying, monitored at 9.00 am and scored if more than half of the ground was covered by snow, was greatest in the Central Highlands, over 60-100 days. The snow did not lie for so long in the North-west Highlands, having a mean of 40-60 days (Met. Office, 1979). These conditions combine to give cool summers, and extended snow cover in winter.

2.3.2: Substratum

McVean (1964) described the immature soils of silt, sand and humus in pockets on the screes. A pH of 4.1 was recorded in humus derived from decaying *Athyrium*. McVean and Ratcliffe (1962) gave a surface pH range of 3-4. The screes are free-draining but supplied by melt-water and seepage to maintain an adequate level of moisture. Odland (1991) found that *A. distentifolium* grew in calcareous conditions in Norway, although still requiring late snowbeds to reduce the competition.

2.4: Communities

McVean and Ratcliffe (1962) closely related the distribution of *A. distentifolium* to length of snow lie. Two types of *A. distentifolium* communities were distinguished:

The first was more basic with rare *Cicerbita alpina*, frequent *Dryopteris expansa*, *D. filix-mas, Rumex acetosa*, *Polystichum lonchitis* and *Sedum rosea*. Grazing pressure has restricted this association to ledges so there are few natural montane meadows and all populations are above the present limit of woodland (McVean, 1964). This community is similar to the fern meadow Odland (1981) described in West Norway with *Cicerbita alpina,* occasional *Phegopteris connectilis*, sterile *Geranium sylvaticum*, *Rumex acetosa* and *Stellaria nemorum*. The closest NVC community to this (Rodwell, 1992) is U16 the *Luzula sylvatica-Vaccinium myrtillus* tall-herb community which includes U16a the *Dryopteris dilatata-Dicranum majus* sub-community. *D. dilatata*, *Gymnocarpium dryopteris*, *Phegopteris connectilis*, *Oreopteris limbosperma*, *Athyrium filix-femina*, *A. distentifolium* and *Dryopteris affinis* are prominent components. While mostly found on less accessible ledges this community is sometimes locally abundant.

The second of McVean and Ratcliffe's communities is an acidic, higher altitude association with abundant *Cryptogramma crispa*, linked to poor soil and low fertility. The NVC classification (Rodwell, 1992) related this second type of *Cryptogramma*

crispa-Athyrium distentifolium snowbed vegetation to U18. The constant species are listed as *Alchemilla alpina*, *Athyrium distentifolium*, *Cryptogramma crispa*, *Deschampsia caespitosa*, *D. flexuosa*, *Galium saxatile*, *Rumex acetosa*, *Saxifraga stellaris*, *Viola palustris*, *Barbilophozia floerkii*, *Hylocomium splendens*, *Hypnum callichroum*, *Kiaeria starkei*, *Polytrichum alpinum*, *Rhytidiadelphus loreus* and *Cladonia bellidiflora*. This community is described as occurring among boulders around the steeper areas behind snowbeds.

Some *A. distentifolium* also occurs in U11, the *Polytrichum sexangulare-Kiaeria starkei* snow-bed community (Rodwell, 1992).

2.5: Response to biotic factors

2.5.1: Grazing

McVean (1964) observed that grazing has restricted the distribution within Scotland of the subalpine meadows which are found elsewhere (Odland, 1981). In 1881 Britten wrote of *A. distentifolium* that it was "much relished by sheep and collectors have said that it is difficult to obtain specimens which have not been cropped by these animals". Moore (1859) recorded that "On the hill sides in exposed places, the fronds are very commonly damaged by winds, spring frosts or by animals, and it is only in the more sheltered localities that perfect specimens can be obtained". In 1928 Adams (1930) noticed that there were fewer plants of *A. distentifolium* in Corrie Ceanne Mor near Braemar than expected and linked this to the recent introduction of a herd of sixty goats able to ascend ledges which had previously not been reached by sheep or deer. In nearby Caenlochan, Cowan (1911) described extensive grazing by deer.

2.5.2: Competition

Overwinter snow provides a specialised habitat which restricts competition (McVean, 1958). Vigorous clumps of *A. distentifolium* shade smaller plants and Gjaerevoll (1950) described the prodigious amounts of litter which suppressed other plant growth.

2.5.3: Human influence

Land management has affected the grazing pressure through control of sheep stocking levels and the amount of deer culling. The habitat is less affected than lowland sites, although *A. distentifolium* would probably have been in the upper zone of woodland (McVean and Ratcliffe, 1962), the ungrazed remnants among boulder screes occupy a semi-natural environment. Remote location, deep winter snow cover and high altitude give protection from recreational disturbance. The upland habitat is, however, very vulnerable to global warming and enhanced deposition of pollutants (3.5).

2.6: Response to adversity

2.6.1: Gregariousness

A. distentifolium can be found in populations varying in size from a few plants to extensive colonies (Rodwell, 1992).

2.6.2: Performance in various habitats

The height of *A. distentifolium* varies over a wide range depending on the degree of exposure and nutrient availability. Several authors have commented on how frequently it was infertile: "In damp gorges and among tumbled rocks it was often destitute of fructification but in more open places it was abundantly in fructification, varying in height from six inches to three feet four inches in height" (15 cm-100 cm) (Backhouse, 1852).

There is a wide range in the reported height of *A. distentifolium*. Odland (1995) recorded frond sizes from 11 cm to over 150 cm. Schaminée *et al.* (1992) measured ferns up to 1 m high in the Massif Central, France. Davis (1965) noted southern European *A. distentifolium* from 2000 to 2700 m that was 20-50 cm high. The North American *A. distentifolium*, var. or subsp. *americanum* has fronds up to 80 cm (Cody and Britten, 1989).

In Norway, Odland (1995) found a correlation between frond size and fertility of *A. distentifolium*. The percentage of fertility showed a steady increase with frond size and beyond 71 cm all fronds were fully fertile. The tallest fronds he recorded were over 150 cm. There was decreasing fertility with increasing altitude and in water stressed areas. The highest percentage of fertile fronds was in subalpine rich talus meadow at 500-900 m. In very late snow beds the fronds were not fertile (Odland, 1991).

2.6.3: Effect of frost, drought, etc.

Plants may be frosted in late spring or early in autumn but there is usually no frost during the short growing season (McVean, 1958). Sato and Saki (1981) used both gametophytes and sporophytes of *A. distentifolium* in freezing experiments which were examined for damage and regrowth. When freezing or defrosting they changed the temperature gradually and the material was thawed at the end of the experiment at 0 °C. Total browning implied complete death. The freezing tolerance was the lowest temperature at which tissues remained alive. They recorded the percent area of gametophyte which was unbrowned and allowed three months for signs of regrowth. It was found that sporophytes could withstand freezing to -15 °C for one day but were killed at -20 °C. However some marginal cells on the wings of some gametophyte were still alive after -70 °C and a gametophyte could regenerate from a single cell. This indicated that overwinter snow cover is necessary to protect from extreme temperatures. By growing in areas with late-melting snow, *A. distentifolium*

plants receive seeping moisture and there are no accounts of performance under drought.

2.7: Morphology

2.7.1: Clones and rhizomes, frond stomatal density

A. distentifolium fronds arise in irregular shuttlecocks from a central crown. The rhizomes can branch and spread through the scree, building up very large clumps (Page, 1982).

There are no published data on *A. distentifolium* stomata.

2.7.2: Mycorrhizas

Three papers which listed subalpine species including *Athyrium distentifolium* were found, all published in Poland. Nespiak (1953) found no mycorrhizas in *Athyrium alpestre* (*A. distentifolium*) in an *Oxyrieto-saxifragetum* association, but this community suggests a high nutrient environment where mycorrhizas might be less beneficial (Read *et al*, 1976). Dominik and Nespiak (1953) gave positive records of arbuscular mycorrhizas from *Picetum mughi* and *Adenostyletum-alliariae* associations. Dominik *et al.* (1954) also noted *A. distentifolium* roots colonised by arbuscular mycorrhizas in a *Picetum excelsae-myrtilletosum* association.

2.7.3: Perennation and vegetative reproduction

No published information was found other than references to large branching rhizomes (Page, 1982; Gjaerevoll, 1950; Odland, 1991).

2.7.4: Chromosomes

Manton (1950) counted the chromosomes of *A. distentifolium*, *A. flexile* and *A. filix-femina* and for all of them found that 2n = 80.

2.7.5: Physiological data

There is very little information published other than temperature responses for the initiation of growth (Odland, 1991; 3.2.3 and 5.3.4), and the response to freezing (Sato and Saki, 1981; 2.6.3, 3.3.3 and 3.3.4).

2.8: Phenology

In Norway, where the snow could last until August, Odland (1995) found that *A. distentifolium* at 750 m usually commenced growth in June. Frond expansion did not start until the soil temperatures at a depth of 5 cm reached 6-7 ° C. It then took 24-27 days for the fronds to fully expand. This was faster than *Oreopteris limbosperma* or *Matteucia struthiopteris* which grew nearby (Odland, 1991). In Central Europe, in the areas of longest snowcover, *A. distentifolium* did not normally emerge until the end of June and growth soon commenced (Schaminée *et al*, 1992).

Sato *et al.* (1989) found that "summer green" ferns like *A. distentifolium* and *A. filix-femina* in Austria were damaged by a late frost or snow fall. The fronds started to die back before sporangia could mature. The spores of Central European *A. distentifolium* are ripe in July or August (Davis, 1965). Sato and Saki (1981) reported that the spore dispersal period of *A. distentifolium* in Japan was only in the first part of September.

2.9: Reproduction

2.9.1: Reproduction of sporophyte, number of fronds and spores

Little information has been published. Scottish plants have been reported to frequently be infertile (Backhouse, 1852) and *A. flexile* is fertile at a smaller size than *A. distentifolium* (Boswell, 1886; Page, 1982). The spores have a folded and ridged perispore giving the appearance of a reticulate surface (Page, 1982).

2.9.2: Discharge and dispersal of spores

Periods given for spore-shedding of *A. distentifolium* vary from July and August (Davis, 1965) to the early part of September only (Sato & Saki, 1981).

2.9.3: Germination of spores

No information available on *A. distentifolium*.

2.9.4: Ecology of the gametophyte

Performance under different degrees of freezing has been recorded in 2.6.3 (Sato and Saki, 1981). Sato (1982) observed that summer green ferns, including *A. distentifolium,* had prothalli that overwintered as gametophytes and may not be fertilised in the first full season. Sporophytes were produced towards the end of the second season and overwintered as very small plants.

2.9.5: Hybrids

The hybrid of *A. filix-femina* and *A. distentifolium* named *Athyrium x reichsteinii* Schneller & Rasbach has been recorded in Europe but not in Britain (Schneller and Rasbach, 1984). The hybrid exists as a diploid, similar in size to the parents, occurring as dense clones which compete well. It is morphologically intermediate with a round sorus and a small, but visible indusium. Most of the spores were abortive and none

germinated. Triploid hybrids also occur, with 120 chromosomes counted from the root tip. These have hybrid vigour and two types were found, resembling one parent or the other. While wild hybrids are not uncommon in Switzerland, an attempt to synthesise hybrids was not successful. There was no suggestion of introgression (Schneller and Rasbach, 1984).

2.10: Grazing

2.10.1: Animals feeders or parasites

Sheep, goats and deer have all been known to eat *A. distentifolium* (Britten, 1881; Moore, 1859; Cowan, 1911; Adams, 1930).

A larva of *Autographa gamma* (Silver-Y moth) was found feeding on *A. distentifolium* but it is unusual to find invertebrates feeding on ferns (McHaffie, 1997a).

2.10.2: Plant parasites

None recorded.

2.11: History

In the immediate postglacial period, from fourteen thousand years ago, snowbed communities would at various times have been widespread at all altitudes. There is no specific mention in pollen diagrams of the distribution of *A. distentifolium* and the associated *Cryptogramma crispa* but the spores of *A. distentifolium* were possibly not very durable and might be included in the frequent references to undifferentiated ferns in Rymer, 1977; Donner, 1958; Moar, 1968 and Webb and Moore, 1982. Populations would gradually have become more isolated as the plants became restricted to a few high altitude sites. Increasing grazing pressure has further restricted the distribution.

The cold period known as the "Little Ice Age" in the 17th and 18th centuries might have marked a return to more suitable conditions for *A. distentifolium*. The range could have been extended and at the end of this period new habitats may have been available on a scale which has not been equalled since the end of the most recent phase of glaciation. Ballantyne (1986) described a comparatively fast-moving lobe of solifluction which he linked to this period and indicated a disruption of the landscape. Present screes have become relatively stable and new habitats are not normally exposed very frequently.

Moore (1859) gave alternative names for *A. distentifolium*, frequently without giving a source. Names quoted for *A. distentifolium* include:

Polypodium rhaeticum pt L. Linné, 1753 (Moore 1859)

Aspidium distentifolium Tausch ex Opiz (according to Steudel, 1841)

Aspidium rhaeticum Swartz, 1800 (Moore, 1859)

Aspidium alpestre Hoppe, 1805 (Moore, 1859)

Polypodium alpestre Hoppe, 1829 (Moore, 1859)

Athyrium alpestre Nylander ex Ledebour (Moore, 1859)

Pseudathyrium alpestre Newman, 1851 (Newman, 1851)

Athyrium alpestre (Hoppe) Rylands (Moore,1859)

Athyrium distentifolium Tausch ex Opiz (Jermy *et al.*, 1978)

2.12: *Athyrium flexile*

2.12.1 Geographical and altitudinal distribution

This taxon has only been recorded in Scotland. The sites range across the central Highlands and except for one doubtful herbarium specimen from Knoydart *A. flexile* has not been found north of the Great Glen. Some of the records are for single plants and there is little information about the abundance in other populations. Many of the site records are untraceable and some, like "Braeriach" and "Glen Einich", may refer to the same site. In all, there are seventeen possible areas where plants have been recorded

(Table 2.1). Glen Prosen is the type locality, first discovered in 1852 (Backhouse) but the plants were rapidly collected out as Boswell-Syme indicated when he wrote that he "had cultivated plants from Glen Prosen, where I believe it is now almost extinct" (Boswell *et al*, 1886). Ben Alder was the most consistently visited location and has remained the only well-known locality. The Royal Botanic Garden Edinburgh herbarium specimen from Corrie an Lochain is wrongly identified, and has not been listed. Two Natural History Museum records for Glen Doll were also mistaken identification and have not been included.

Table 2.1 Records for *Athyrium flexile*. The source of the record is given as in the BRC lists, references in journals and herbarium specimens in herbaria, together with the date and collector or author, if known. Abbreviation: BM (Natural History Museum, London). OX (Oxford). CGE (Cambridge). RBGE (Royal Botanic Garden, Edinburgh). BSE (Botanical Society of Edinburgh). BRC (Biological Records Centre). Aberdeen (University Herbarium). Dates given as in source.

Date	Source	Locality
July 1950	BM	Ladhar Bheinn, **Loch Hourn**
Aug 1911	Perth Museum	**Meall nan Tarmachan**
1919	OXF	Meall nan Tarmachan
23 07 1920	BM	(King's Seat? same square reference)
16 07 1974	CGE	**Beinn an Dothaidh**,
July 1943	Mackechnie & Wallace, RBGE	**Meall Buidhe** above Crannach
1886	Watson & Macfarlane, Proceedings B.S.E	Ben Creachan **Beinn a' Chreachain**
Oct 1889	Haggart, Perth Museum	near Ben Cruban (Beinn a' Chreachain?)
1898	White, Flora of Perthshire	Glen Lyon near Ben Cruban
1891	OXF	**Aonach Mor**
1904	Cowan, Proceedings B.S.E	**Ben Nevis** 1 plant
03 08 1883	BM	**Caenlochan**
1911	Cowan, Proceedings B.S.E	Caenlochan
24 07 1852	BM	**Glen Prosen**
1853	Newman, The Phytologist,	Glen Prosen
1853	BM	Glen Prosen
1855	Backhouse, RBGE	Glen Prosen
1855	OXF	Glen Prosen, head of
1861	Moore, RBGE	Glen Prosen

July 1866	Aberdeen	Maire Burn Glen Prosen
July 1867	BM	Glen Prosen
1959	BM	**Lochnagar**, W corrie
Aug 1898	BM x 2	**Cairn Gorm**
July 1888	OXF	**Braeriach**
Aug 1888	OXF	**Glen Einich**
1973	BRC	Glen Einich, Corrie Dhondail
1891	OXF	**Glen Avon**
1990	Tennant, RBGE	**Loch Etchachan**
1993	Tennant, BM	**Derry Cairngorm**
1867	Balfour, RBGE	**Ben Aulder**
07 08 1867	BM	Ben Alder
Aug 1867	CGE	Ben Alder
Aug 1867	Fraser, RBGE	Ben Aulder
Aug 1867	Craig-Christie, RBGE	Ben Aulder
1867	J. H. Balfour, Pros BSE	Ben Aulder
Aug 1875	BM	Ben Alder
Aug 1875	White, Perth Museum	Ben Aulder
1879	Aberdeen	Ben Aulder
Sept 1880	White, Perth Museum	Ben Aulder
July 1887	Aberdeen	Ben Aulder
Aug 1946	Sledge, RBGE	Ben Alder, North Corrie
12 08 1946	BM	Ben Alder, N Corrie
11 08 1947	BM	Ben Alder, N Corrie
Aug 1971	BRC	Ben Alder, Garbh Choire
Aug 1971	BRC	**Ben Alder, Garbh Choire (Beag)**
July 1973	McCallum-Webster, RBGE	Ben Alder, Garbh Corrie 27/47
16 08 1957	Ratcliffe, RBGE	**Ben Eibhinn**, Ardverikie Forrest
1887	Craig, Proceedings BSE,	Possibly Creag Meagaidh

2.12.2: Habitat

A. flexile has always been found with *A. distentifolium* and requires similar conditions of late snow lie (Page, 1982).

2.12.3: Communities

The NVC classification of the *Cryptogramma crispa-Athyrium distentifolium* community, U18 (Rodwell, 1992) mentions the very local occurrence of *A. flexile*.

2.12.4: Response to adversity

a) Gregariousness

The records of *A. flexile* (Table 2.1) vary from specific references to single plants as at Beinn an Dothaidh (Stirling, pers. comm, 1995) and Ben Nevis (Cowan, 1904) to indications of a larger population, as in the reference to the original Glen Prosen site where *A. flexile* was found "in some quantity" (Moore, 1859).

b) Performance in various habitats

A. flexile is a smaller plant but in contrast with *A. distentifolium* is usually fertile. Observations at Ben Alder indicated its precocious fertility: "Mr A. C. Christie tell me that *A. flexile* fruits when only three inches long and *A. alpestre* growing with it not under nine or ten inches" (7.5 cm and 23-25.6 cm respectively), (Boswell *et al*, 1886). Page (1982) noted that fronds of *A. distentifolium* are rarely fertile below 15-25 cm in comparison with *A. flexile*.

2.12.5: History

An outline of the discovery of this taxon has already been given (1.2).

Names quoted by various authors include:

Pseudathyrium flexile Newman (Newman, 1853)

Polypodium alpestre var. *flexile* Moore (Moore, 1859)

Also gives *Athyrium alpestre* var. *flexile* (Moore, 1859)

Polypodium flexile (Newman) T. Moore, no Fée (Dandy, 1958)

Athyrium alpestre var. *flexile* Milde (Milde, 1867)

Athyrium alpestre subsp. II *flexile* (Boswell *et al*, 1886)

Athyrium flexile Syme (G. C. Druce, 1932)

Athyrium alpestre var. *flexile* (Newman) Druce (C,T & W, 1952)

Athyrium alpestre var. *flexile* (Newman) Milde (C,T & W, 1962)

Athyrium distentifolium Tausch ex Opiz var. *flexile* (Newman) Jermy (Jermy, 1978)

Athyrium flexile (Newman) Druce (Page, 1982)

CHAPTER THREE Distribution and the response to the environment of *A. distentifolium* and *A. flexile*

3.1: Introduction

This chapter examines the habitats in which *A. flexile* and *A. distentifolium* were found. Data were collected at the field sites to define the physical environment and the different communities in which both taxa occur. Ecological differences were sought between habitats in which both *A. flexile* and *A. distentifolium* occur compared with sites exclusively occupied by *A. distentifolium*. Former localities were visited to look for *A. flexile*. These localities had been traced through herbarium specimens, from records sent to the BRC and from references in journals (2.12, Table 2.1). Two sites, Glen Prosen and Bridge of Orchy, were visited at regular intervals throughout the growing season for the three years 1995-1997 to take regular measurements and make observations on the phenology of the two taxa. Temperature was recorded at these two localities to supplement published information and this provides a baseline for an assessment of the effects of climate change. Other areas were visited on at least one occasion to look for *A. flexile*. Some exclusively *A. distentifolium* habitats, particularly in the North-west, were visited to suggest comparisons with *A. flexile* sites. An experimental component was necessary to supplement field observations on *A. distentifolium* and *A. flexile*'s response, both as sporophytes and gametophytes, to desiccation and freezing. Experiments are described which tested these taxa's response to extremes of temperature and to desiccation.

3.2: The Habitat

3.2.1: Site descriptions

Glen Prosen was the original type locality found in 1852 by J. & J. Backhouse with T. Westcombe (Backhouse, 1852). The site was collected to near extinction within four decades (Boswell *et al*, 1886). A small population, which seemed to answer to a

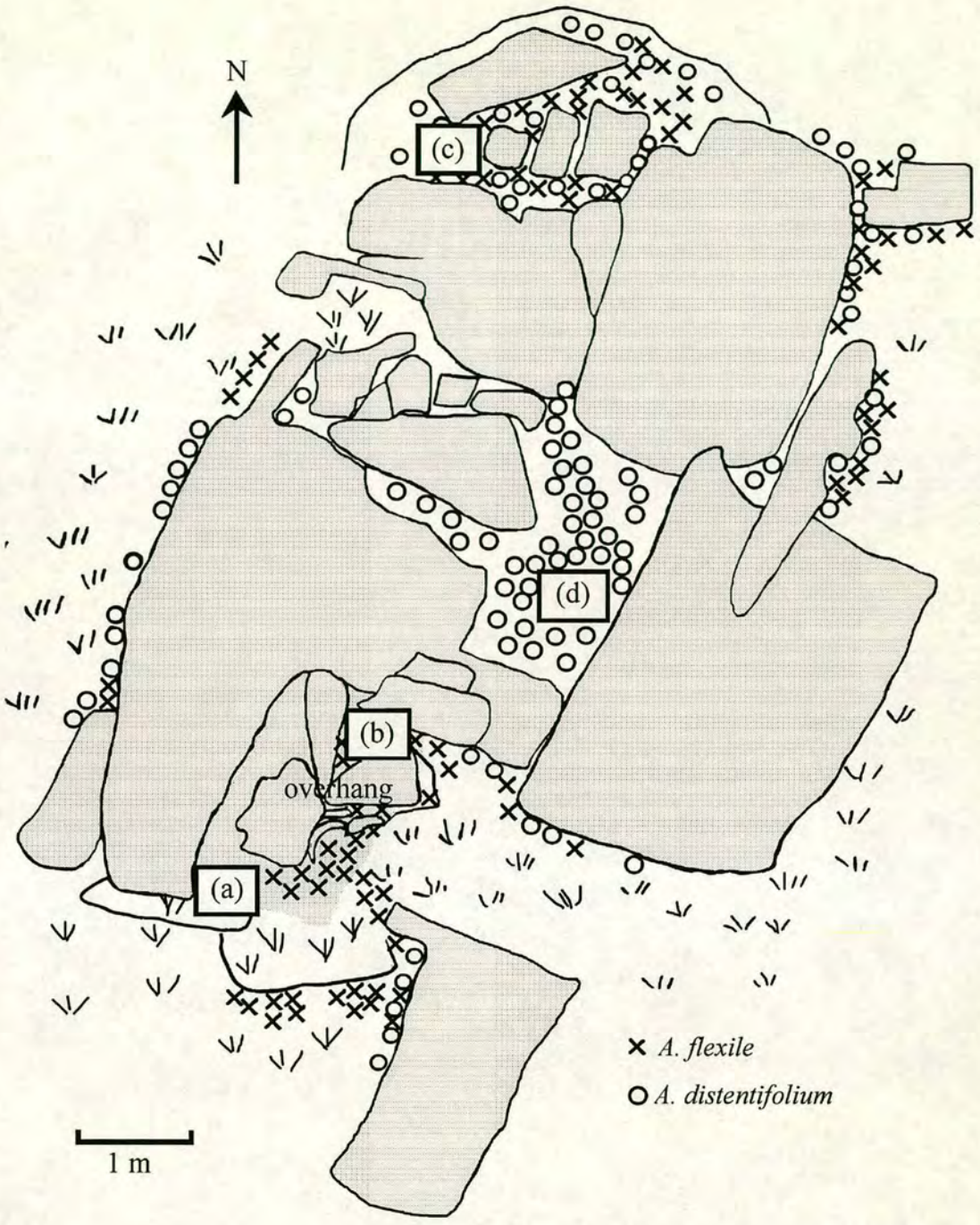


Figure 3.1 Plan of the site at Glen Prosen, on a south-facing slope, showing the approximate positions of each taxon. The positions of the four thermisters (a-d) are indicated. They were linked to a data-logger situated between (a) and (b) under the overhanging rock.

similar description, was found in 1995 (Figure 3.1) (McHaffie, 1997b). As this was more accessible than most of the other localities, it was used as one of the two field study sites. This population of *A. flexile* and *A. distentifolium* grow in and around large slabby boulders. The whole site is nearly 12 metres across overall. The slope is south-facing, approximately 45°, at 800 m, backing onto a rocky outcrop which retains overwinter snow. Below the rocks there is a flushed area with a small stream during wetter periods. The *A. flexile* plants are all found around the perimeter of the rocks under the edges of the slabs. They are less than 30 cm high, often as little as 10 cm, and intermixed with infertile *A. distentifolium* and some *Dryopteris expansa*. In the centre of the rocks are massive clumps of *A. distentifolium* that can grow up to 90 cm, and approximately half of these plants are fertile. There is no vegetation immediately under the dense fronds where there is thick litter. *A. flexile* was originally described as growing "in some quantity" (Moore, 1859) and it might have extended further down the slope. Two more clumps were found, one only 20 m down the slope and another near the foot, at 750 m a.s.l.

Ben Alder, 85 km due west of Glen Prosen was first found as an *A. flexile* site in 1867 (Balfour, RBGE, 1867) and was frequently visited thereafter. The Great Corrie, Garbh Choire, has an eastern aspect with a greater concentration of ferns on the north and east-facing sides, well above 800 m. The Corrie retains snow over a long period and in 1994 there were still substantial accumulations on the floor of the corrie in August, with *Cryptogramma crispa* only just emerging from beneath the late lying snow. Most of the plants of both *A. distentifolium and A. flexile* are small, usually less than 40 cm. The topography varies between very large boulder scree and more accessible areas of smaller rock-size. The slope is 40-45° with 15-80% of the surface covered with rock (Mean 50% SE 11.7). An additional locality for *A. flexile* at 850 m was found nearby in 1971 (BRC) in the Garbh Choire Beag among rocks close below the steep cliffs. This site was revisited in 1997 and *A. flexile* was found sporadically along the base of the cliffs.

Beinn Eibhinn was first found in 1957 (Herb. Ratcliffe, RBGE). It is on the west side of the Ben Alder range and only 5 km from the Ben Alder population. The corrie has a north-east facing aspect, 5-45°, and all the ferns are small, usually less than 30 cm. The scree varies in size from loose fragments, which are unstable, to large boulders. The ferns are found above 900 m. Most of the ferns grow between rocks that occupy up to 90% of the surface. The slope is 35-45°.

Creag Meagaidh near Loch Laggan and 18 km north west from Ben Alder has large populations of vigorous *A. distentifolium*. Four clumps of *A. flexile* have been found, which implied an extended search might locate more. The *A. flexile* clumps were found above 800 m on a north-facing slope in the smaller corrie above Corrie Ardair. The slope is reasonably stable and has well vegetated strips alternating with scree at 45°. Three clumps grow in an area that appears to have slumped down and has a horse-shoe-shaped lobe of boulders below the rocky outcrops. This created a small area protected by steep cliffs above and large boulders below. This habitat might have been created in the cooler period of the Little Ice Age. An old record of *A. flexile* from Glen Spean (Craig, 1887) could relate to this area as this is one of the localities that was visited.

The range of hills at **Bridge of Orchy** 30 km south west from Ben Alder include Beinn an Dothaidh, Beinn Achaladair and Beinn a' Chreachain and offer several possible sites. Although there are two former records on Beinn an Dothaidh, (1974, 1978, CGE), these were for single plants and despite repeated attempts, they have not been refound. A large population exists between Meall Buidhe and Beinn Achaladair, first recorded in 1943 (Mackechnie and Wallace, RBGE). The *A. flexile* here is larger than at any other site, some are nearly 60 cm tall, and almost as large as the *A. distentifolium*. The Corrie is north facing but the main population of *A. flexile* has a north-west aspect, 300-355° E of N, and is found from 700 m upwards. The slope is 40-45° with rock cover ranging from 20-90% (Mean 54% SE 5.4). The monitored plants were within metres of one another at three locations (marked with arrows, Figure 3.2). The first area for monitored plants was in more open scree, which

emerged from the snow before the other two locations. The second was in a v-shaped scree where the snow lay two metres deep in May 1996. The third was a more extensive v-shaped scree with larger boulders, some several metres across.

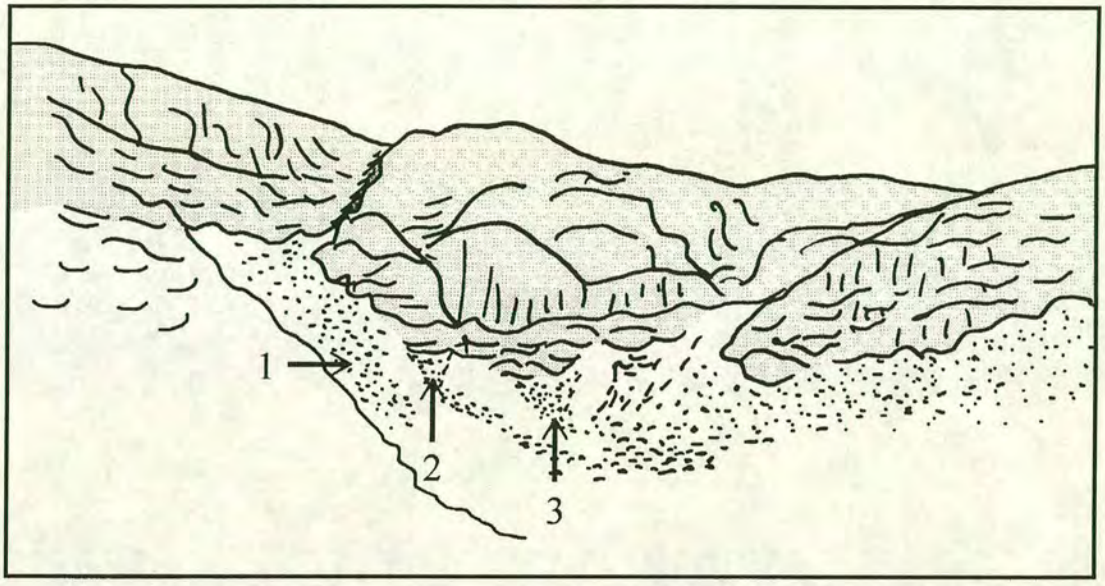


Figure 3.2 Sketch of the north-west facing side of the corrie at Bridge of Orchy between Meall Buidhe and Beinn Achaladair. Approximately 1 km is in view. There are steep cliffs above and a stream on the left of the fern beds. The extensive boulder screes (dotted) have mixed populations of *A. distentifolium* and *A. flexile*, with *A. flexile* more frequent in the centre of the site. The arrows mark the areas where roots were collected to determine the presence of mycorrhizas (4.9) and where the monitored plants were located (5.5).

A further small population of *A. flexile* was found below Beinn a' Chreachain one km to the north-east. Plants were recorded in a "large north-facing corrie" on Ben Creachan at the head of Glen Lyon in 1885 (Watson and Macfarlane, 1886) and these Beinn a' Chreachain ferns are possibly in the same place. One clump of *A. flexile* grows by the edge of the lochan at 700 m that is 300 m away from the other seven clumps growing at 750 m. These face due north on a 30° slope.

The **Cairngorms** have many records of single plants. Some, like the reference to Glen Avon, 1891 (Oxford) or Cairngorms, 1898 (BM) are too imprecise to relocate. There are two more recent records for single plants near Loch Etchachan, 1990 and 1993 (Tennant, RBGE & BM). There is a record from **Lochnagar**, west corrie, 1959

(Somerville, BM) but the Corrie an Lochain, 1973 (RGBE) specimen is not satisfactory and must be discarded. **Corrie Dhondail** in Glen Einich has two records in 1888 (Oxford), and 1973 (BM). Braeriach 1888 (Oxford) may relate to the same locality. Two records from widely spaced intervals suggested a reasonable population from Corrie Dhondail but the corrie has few habitats for ferns. The whole corrie is filled with deep, angular scree and vegetation is only found on or below the cliffs. In 1997 two clumps of *A. flexile* were found close together, growing through the scree at 900 m, facing north, with no associated vegetation. This site is 40 km north west from Glen Prosen.

There are three records from Meall nan Tarmachan, specifically mentioning King's Seat and Killin: 1911 (Perth), 1919 (Oxford), 1920 (BM). Nothing was found at Meall nan Tarmachan in July 1995 but precise locations were lacking.

Two single plants were recorded from Aonach Mor, 1891 (Oxford) and Ben Nevis, 1903 (Cowan, 1904). Caenlochan has an extensive population of *A. distentifolium* and there are two records of *A. flexile*: 1883 (BM), 1910 (Cowan, 1911). Two BM records for Clova (1964) and Glen Doll (1968) were misidentifications.

Ladhar Bheinn, (1950, BM) on Loch Hourn, is the only record of *A. flexile* north of the Great Glen. On the sheet of herbarium specimens only one frond resembled *A. flexile*. During a visit in 1997 no *A. flexile* was found. If this taxon is present the populations are not large. Some of the *A. distentifolium* had pinnule division which resembled *A. flexile*. The lower cliffs were extensively grazed but large, dense swards of *A. distentifolium* were found on cliff ledges and less accessible areas.

The occurrence of occasional plants indicate that *A. flexile* can be found across a wide part of the Central Highlands and there are possibly still populations to be located. Growing as they do in acidic areas of less general botanic interest they are less likely to be found than plants in more frequented basic areas.

3.2.2: Variations in the weather over three field seasons 1995-97

During the three years of this study there was considerable variation in the seasons. In 1995, at Bridge of Orchy, there was moderately late-lying snow which did not finally disappear from some *A. distentifolium* plants until July. *Cryptogramma crispa* was the last to be uncovered in late July as the longest-lying snow was on the side of the corrie where only the *Cryptogramma* grew. In 1994 it had been observed at Ben Alder that *C. crispa* was still emerging from the snow in August. The 1995 summer was very hot and dry. At Bridge of Orchy the screes were irrigated by the remnants of snow still melting from high above, and water seeping from the cliffs. At Glen Prosen and Ben Alder there were some areas which dried out and plants were noticeably stressed and shrivelled in July and August. The Glen Prosen plants which had been affected were re-examined the following year and had recovered, but were not fertile.

In the winter of 1996 the main snowfall occurred with prevailing winds from a different direction than usual. At Bridge of Orchy in 1995 the bulk of the snow had been on the north-east facing side of the corrie, but in 1996 it was on the north-west facing side. Some of the Bridge of Orchy plants had no snow cover by May, but growth did not begin for all the plants until mid June. At the beginning of June some plants were still beneath at least a metre of snow. The Glen Prosen plants on a south-facing slope were uncovered by early May but only started to expand at the beginning of June. The 1996 summer was more typical, with mixed weather and there was no lack of water.

In the winter of 1996-97 there was very little snow and on the first visit at the end of April there was no snow lying on either of the field sites. The plants at Glen Prosen started to grow in a very mild spell in early May, but were severely frosted. The Bridge of Orchy plants had not started to grow and successfully flushed from the beginning of June, by which time the Glen Prosen ones had started again. This was another damp summer, which might have helped to compensate for the lack of meltwater. However, the Cairngorms lacked the usual large snow beds to provide

seeping water which is a necessary part of the habitat in the drier eastern areas. Glen Einich in the Cairngorms had some very stressed plants of *A. distentifolium* in August. They were browned and shrivelled with some newly expanded fronds. This might have been the results of drought. There had been little precipitation and no melting snowbeds. Alternatively the damage could have been due to frost, searing winds, or the effects of pollution (3.5).

Each season was so different it is difficult to generalise about the habitat, but some common features emerge. The higher areas on a slope tend to have later-lying snow and the most frequent fern is *Cryptogramma crispa*. The bottom of a slope, is flatter, the ground is more grazed and there are few ferns. At Ben Alder this area in the main corrie often had thick snow, when it had melted from intermediate areas but was still present high on the back wall of the corrie. This corresponded to the observations of Geiger (1965). As cold air flows downhill, concave surfaces are coldest at night, giving frost hollows. During the day the wind blows up slope so it is cold in the valley bottom and cold around the high tops but there is a warmer thermal belt of varying altitudes in the middle which is the first to be free of snow. This may help to determine the position of specific communities in relation to the surrounding topography.

Snow provides insulation from the air temperature and ground frost during the winter. It also has reflective properties which maintain a cool environment as 75-95% of short-wave or solar radiation is reflected back from snow, but this proportion declines as the snow gets older and dirtier. Trees or rocks beside the snow absorb short wave radiation and warm up, emitting long-wave or heat energy. This is seen as the snow melts first against the foot of a cliff or very large rocks. Snow emits long wave radiation and with a clear sky at night there is a cold layer of air immediately at the snow surface. As spring approaches, the layer of cold air causes a time lag in the melting of the snow (Marchand, 1987). This delaying mechanism might help to prevent premature growth.

Accumulations of several metres of snow can melt rapidly in the right conditions. Geiger (1965) established that snow melts faster with higher humidity. Evaporation only accounts for 0.2-1.0 mm per day while rain melts snow eight times faster than evaporation. However, mist or fog is even more effective than rain, as water vapour condensing on the surface of the snow releases heat (Marchand, 1987).

One frequent observation while on fieldwork was the prevalence of hill cloud. Friend and Woodward (1990) noted that in maritime mountain ranges there is a decline in sunshine duration with altitude. Increased cloud cover offers good conditions for melting snow and also helps to maintain a more even temperature, particularly at night.

Another temperature control is derived from the immediate environment where a scree slope provides a local micro-climate. Grace and Unsworth (1988) reported higher temperatures on a scree than any other area on a hillside. With an air temperature of 20 °C, a patch of *Pteridium aquilinum* was 21.1 °C, *Calluna vulgaris* was 22.5 °C but a dry scree was 30 °C. The mass of rock might also be expected to maintain a more even temperature continuing overnight.

3.2.3: Temperatures

A data logger recorded temperatures at four points (Figure 3.1) around the rocks at Glen Prosen during 1996 (Figures 3.3a-d). A short sequence was also recorded from one thermister at the same site in 1997 (Figure 3.4) together with screened maximum and minimum thermometer readings at fortnightly intervals (Table 3.1). Readings were also taken from a maximum and minimum thermometer at Bridge of Orchy overwinter 1996-97 and during the summer of 1997 (Table 3.2).

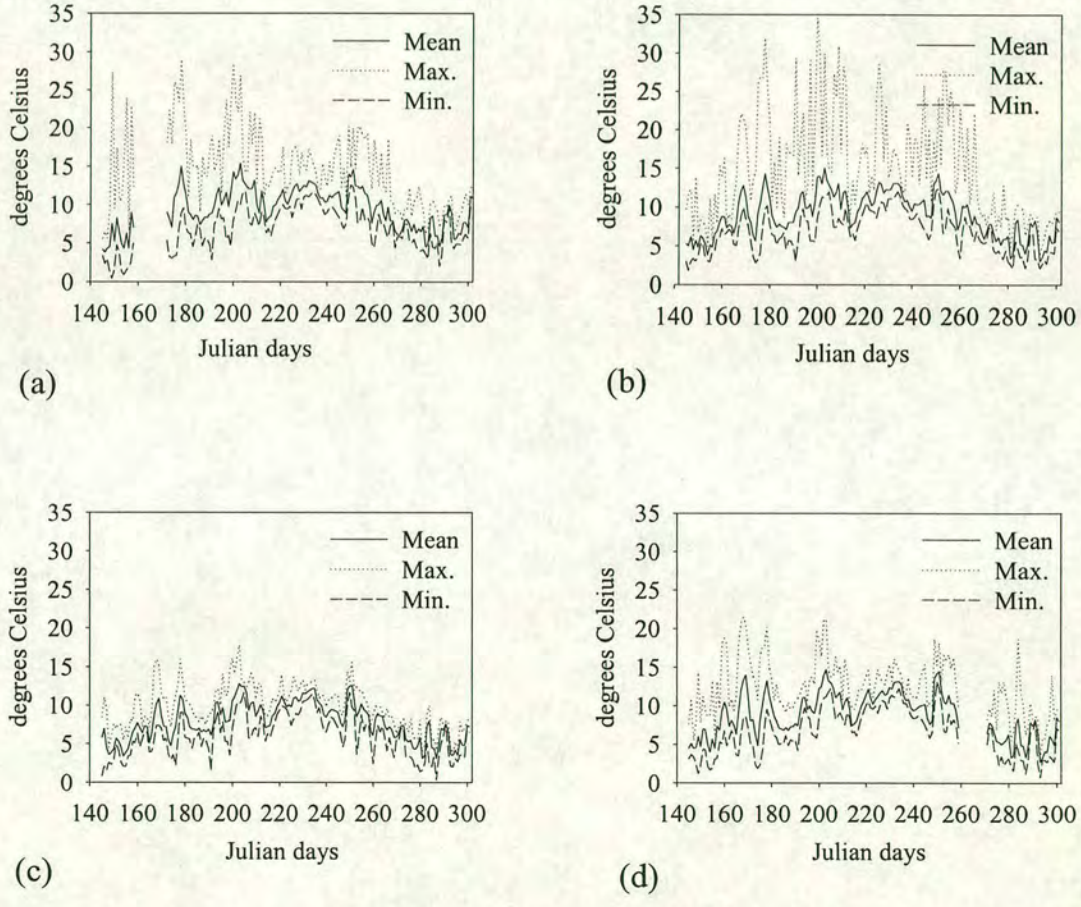


Figure 3.3 Ground temperatures recorded by a data-logger between May 24th and October 24th 1996 on four thermisters placed around the rocks at Glen Prosen. (a) and (b) were placed on the south-facing front of the slope and showed a wider range than (c) located on the shaded north side and (d) in the middle of a large clump of *A. distentifolium.* Locations of the thermisters are shown in Figure 3.1.

Means of the four temperature readings for the period from 19th June to 11th September 1996 when most active above-ground growth occurred (Table 3.1), showed broad similarities, with the mean temperatures ranging from 9.3-11.0 °C. The area in front of the rocks experienced high maximum temperatures in the early part of the season. There was a break in the recording when two thermisters (Figure 3.3a and 3.3d) were removed from the screening. The toothmarks on the cable suggested that this was done by a mountain hare. In the sheltered area under the large rock, temperatures up to 35 °C were reached (Figure 3.3b). At the back of the rocks a more even temperature was maintained without these extremes (Figure 3.3c). The fourth thermister among a large clump of *A. distentifolium* also showed less variation (Figure 3.3d). No frost was recorded during the growing season. The mean temperatures were not high but some of the plants experienced wide fluctuations in temperature. The slope in front of the rocks reached 25-30 °C while night temperatures fell to below 5 °C.

Table 3.1 Means of the maximum, minimum and mean temperatures at Glen Prosen for the period 19th June to 11th September 1996 for the screened thermisters a-d placed on the ground at points around the rocks. Locations shown in Figure 3.1 (± standard error).

Thermister	Mean	Maximum	Minimum
a) Front of rocks	11.00 °C ± 0.2	16.94 °C ± 0.46	8.18 °C ± 0.25
b) Below big rock	10.71 °C ± 0.23	18.73 °C ± 0.7	8.16 °C ± 0.25
c) Back of rocks	9.32 °C ± 0.21	11.50 °C ± 0.23	7.35 °C ± 0.25
d) Clump of ferns	10.29 °C ± 0.23	13.55 °C ± 0.31	8.19 °C ± 0.26

A similar picture emerged in the wide ranging temperatures recorded on the second thermister (b), below the big rock, over a shorter period in 1997 (Table 3.2) (Figure 3.4).

Table 3.2 Means of the maximum, minimum and mean temperatures at Glen Prosen for the period 18th June to 29th July 1997 for Thermister (b) placed beneath the big rock (± standard error).

Thermister	Mean	Maximum	Minimum
b) Below big rock	10.40 ± 0.47	16.09 ± 1.07	8.54 ± 0.43

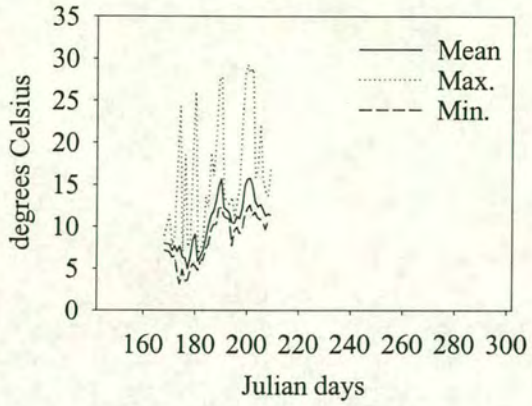


Figure 3.4 Glen Prosen Thermister (b). Temperatures recorded under the big rock on the south side of the site during six weeks from June 18th to July 29th 1997.

In 1997, at the beginning of May, the weather generally was very mild and this stimulated the fronds to grow at Glen Prosen and they were completely frosted. The frost was indicated by the -3 °C recorded on the 13th of May (Table 3.3). As in 1996, the growing season temperatures did not otherwise fall below zero. The maximum temperatures were not as high as at Bridge of Orchy (Table 3.4), which is unexpected on a south-facing slope. However, during field visits the cloud was frequently below the level of the site, which presumably offset the effect of the southern aspect.

Table 3.3 Glen Prosen Temperatures 1997 recorded at 2 weekly intervals on a maximum and minimum screened thermometer

Date	Maximum	Minimum
29.4.97 to 13.5.1997	16 °C	-3 °C
4.6.1997	16 °C	2 °C
18.6.1997	12 °C	4 °C
2.7.1997	11 °C	1 °C
16.7.1997	17 °C	5 °C
30.7.1997	20 °C	5 °C

The minimum overwinter temperature of -4 °C at Bridge of Orchy (Table 3.4) showed that the plants were not required to tolerate very low extremes.

Table 3.4 Bridge of Orchy temperatures 1996-1997 recorded at 2 weekly intervals on a maximum and minimum screened thermometer

Date	Maximum	Minimum
28.10.1996 to		
28.4.1997	15 °C	- 4 °C
12.5.1997	18 °C	- 2 °C
3.6.1997	25 °C	1 °C
17.6.1997	21 °C	4 °C
1.7.1997	21°C	3 °C
15.7.1997	22°C	5 °C
29.7.1997	24 °C	7 °C
17.10.1997	22°C	0 °C

Presumably, as soon as temperatures fell, the accompanying precipitation provided an insulating layer of snow. Frond growth only began in June once the night temperatures rose above freezing. In October the fronds had nearly died down, but even then, only light frosts had occurred. It was observed that *Pteridium* on lower ground showed signs of frosting both in 1996 and 1997 before the ferns in the corrie showed signs of frost. This may indicate the advantage of growing on a slope with drainage of cold air.

Meteorological records for 1941-1970 (Met. Office, 1979) gave a July mean maximum temperature for the Central Highlands, (corrected for 800 m), as 12.4 °C, with a mean minimum of 6.0 °C. These temperatures are lower than the data logger temperatures spanning this period (Table 3.1), but are presumably based on the standard readings at 1.25 m, and the surface of the ground may be warmer.

3.2.4: Precipitation and snow lie

The high altitude of all the field sites ensures a high precipitation. The Average Annual Rainfall Map (Met Office, 1977) for the period 1941-1970 provided mean annual rainfall for each of the *A. flexile* localities. The Glen Prosen site in the east had the lowest mean rainfall of 1600 mm. Areas within the Cairngorms received up to 2000 mm and the Glen Einich area with Corrie Dhondail would have received this amount. Ben Alder, Beinn Eibhinn and Creag Meagaidh further west all received at

least 2000 mm annually. The Bridge of Orchy location, furthest west, had over 2200 mm. Birse and Robertson (1970) compiled a map of exposure and accumulated frost from a synthesis of wind and temperature measurements, together with estimates of exposure damage to the vegetation on a wide range of sites. This showed the field sites in the most extreme category of climatic types in Scotland: "extremely exposed with extremely severe winters". Most of these areas are in the Cairngorms, including Corrie Dhondail. Ben Alder, Beinn Eibhinn and Creag Meagaidh are distinctive in their own range of hills as they stand out in the extreme category. Glen Prosen is on the edge of the second most severe category: "extremely exposed with very severe winters", as is the Bridge of Orchy range of hills. Other hills experiencing equal severity are around Ben Nevis and there are smaller areas north of the Great Glen, most notably around Glen Orrin.

All the sites of *A. flexile* seen during the study period are included within the part of the Central Highlands where snow lies for more than 60 days each year, and often for more than 100 days (Figure 3.5). The ferns typically grow in hollows that trap the snow and are not found on the most exposed ridges. The North-west Highlands, where *A. distentifolium* can be found in abundance, does not have such long snow cover, as mean snow lie does not exceed 60 days annually, although the precipitation is high (Met. Office 1979). The ferns at Glen Prosen that started to grow prematurely in 1997 illustrated the need for the protection of snowcover and the role it plays in controlling excessively early growth. Sato and Saki's work (1981) on *A. distentifolium* sporophytes that could tolerate -15 °C for one day but were killed at -20 °C indicated that the crowns themselves require protection, particularly as they grow in such exposed and extreme areas. Below snow cover a more even temperature is maintained. Marchand (1987) established that once below 40-50 cm of snow, small changes in density of the snow are unimportant and the temperature is almost constant. Odland (1995) in Norway recorded a temperature of 0.3 °C five centimetres below the soil surface, beneath 40 cm of snow. Similarly Kudo (1991) monitored the temperature four centimetres below the surface and found it fell to

0 °C once there was snow cover. Without snow, far greater extremes of temperature are experienced. Holway and Ward (1965) used maximum and minimum thermometers over winter in Northern Colorado. Thermometers which were snow covered had an overwinter maximum of 2 °C and minimum of -8 °C. Areas with little snow cover went down to -30 °C and up to 12 °C.

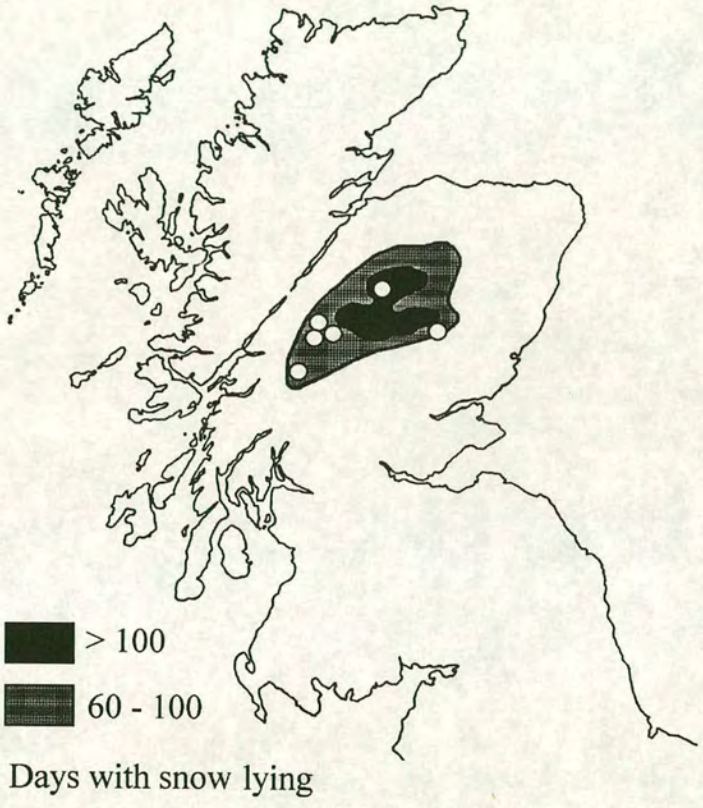


Figure 3.5 Map of Scotland showing the six field sites which are described in the text within the only area in Scotland where snow lies longer than 60 days each year (After Page, 1982). Glen Prosen is the most easterly location, Glen Einich, in the Cairngorms, is the furthermost north. Ben Alder, Beinn Eibhinn and Creag Meagaidh are relatively close together, while the Bridge of Orchy site is the furthermost south and west.

3.2.5: Substrate

Rock

Plants of both taxa in the Cairngorms grow on granite. At Creag Meagaidh, Ben Alder and Beinn Eibhinn the rocks are part of the Moine series, which is mostly acidic. Beinn a' Chreachain, Meall Buidhe and Beinn an Dothaidh near Bridge of Orchy are on Dalradian metamorphosed rocks of sedimentary origin, including limestone. The Glen Prosen rocks are quartzose mica-schist, also Dalradian (IGS, 1977; IGS, 1979; BGS, 1987)

Soil

Soil samples for pH measurements were collected at different times through the season. The Glen Prosen samples were collected within a radius of up to fifteen metres. The Bridge of Orchy samples were collected across the monitored site at intervals ranging from 20-50 metres. They were taken from as near the surface as possible, but this varied considerably. Some samples had more mineral soil than others, which were almost entirely composed of decaying fronds. Once removed from the field they were stored at 4 °C and tested within 48 hours. Five grams of soil were added to 12.5 ml of distilled water, thoroughly stirred and left for 30 minutes before testing with a calibrated pH meter.

Seasonal variation was found, as the Bridge of Orchy values (Table 3.5) showed a slight rise from May to August. The Glen Prosen pH values (Table 3.5) showed a similar rise. Samples were taken in the centre and margin of the Glen Prosen site and also on the adjacent moor. There was little difference between these areas. All the values from different sites including Ben Alder and Creag Meagaidh (Table 3.5), were collected at different seasons and fell approximately within pH 3.5-4.5 and indicated that these sites are generally acidic. Seasonal variation in pH has been recorded elsewhere as Morecroft, Marrs and Woodward (1992) found the pH on Ben Vorlich was lower in June than September. They also found that the available nitrogen was lowest in September, and highest in October when decomposition began again.

Table 3.5 pH samples from four localities collected between May and September

Location	Date collected	Size of sample	Mean	SE	Range
Bridge of Orchy					
	21.5.96	10	3.76	0.08	3.41-4.11
	17.6.97	10	4.06	0.04	3.81-4.24
	16.8.95	8	4.43	0.11	3.98-4.79
Glen Prosen					
edge of rocks	4.6.97	5	3.67	0.06	3.52-3.81
centre of rocks	4.6.97	5	3.43	0.06	3.26-3.57
edge of rocks	13.7.95	4	3.72	0.07	3.53-3.87
edge of rocks	15.9.95	10	4.14	0.02	4.04-4.28
centre of rocks	15.9.95	5	4.49	0.23	4.06-4.94
adjacent moorland	15.9.95	8	4.53	0.09	4.08-4.83
Creag Meagaidh	31.8.95	3	4.45	0.005	4.45-4.46
Ben Alder	8.8.95	6	4.04	0.02	3.97-4.10

3.3: Communities

3.3.1: Associations

Although each population is within a snowbed community there was some variation in the species present at different localities. Twenty-seven 1 m^2 quadrats from the field sites were recorded around *Athyrium* plants which were under observation, and used to compare with the National Vegetation Classification (Rodwell, 1992), communities defined by McVean and Ratcliffe (1962) and a comprehensive analysis of Norwegian stands of *A. distentifolium* (Odland, 1995). Species which only occurred once were omitted. A TWINSPAN (Hill, 1979) ordination recognised three main divisions in the vegetation. There is considerable overlap between the divisions and the most frequent species in all three divisions were *Oxalis acetosella*, *Barbilophozia floerkei*, *Kiaeria starkei, Athyrium distentifolium*, *Vaccinium myrtillus*, *Polytrichum alpinum*, *Galium saxatile, Nardus stricta* and *Rhytidiadelphus loreus* (Table 3.6). Some species overlap between divisions one and two, others between divisions two and three.

Table 3.6 TWINSPAN ordination arranged by frequency and divided into three groups. The source of the individual quadrats are identified by Bridge of Orchy (O), Ben Alder (A), Beinn Eibhinn (E), Creag Meagaidh (M) and Glen Prosen (P). (Names as in Stace, 1997 & Smith, 1978).

	A	A	A	A	A	O	A	E	O	O	O	O	O	O	O	O	O	M	M	O	M	O	O	O	P	P
Racom lanu	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Diplopyhll albic.	1	1	1	1	1	-	1	1	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-
Kiaeria falcata	-	-	1	1	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hylo. umbratum	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Dicranum majus	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Agrostis capill	-	-	-	1	1	1	1	-	1	1	1	1	1	-	1	-	1	1	1	-	-	1	-	-	-	-
Sphagnum cap	-	1	1	-	1	-	1	1	1	-	-	-	1	1	-	1	-	1	1	1	1	1	-	-	-	-
Descamp ces	-	-	-	1	1	-	-	1	-	-	-	-	-	-	-	-	1	1	1	1	1	1	-	-	-	-
Racom hetero	-	-	-	-	-	-	-	1	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Huperzia selago	1	-	-	1	-	-	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Crypto. crispa	-	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	1	-	1
Oxal. acetosella	1	1	1	1	1	-	-	-	-	-	-	1	1	1	1	-	-	-	1	1	-	-	-	-	1	1
Barbiloph. flo	1	1	-	1	1	1	-	-	1	-	1	1	1	-	1	-	-	-	-	1	-	-	1	1	-	-
Kiaeria starkei	1	1	1	-	-	1	-	1	1	1	-	-	1	-	1	-	-	-	-	-	1	-	-	1	-	-
A. distentifolium	1	1	1	1	-	1	1	1	1	1	-	1	1	1	1	1	1	1	-	-	-	-	1	1	1	-	1
Vaccin. myrtillus	-	-	-	1	1	1	1	1	1	1	1	-	-	-	1	-	-	-	1	-	-	1	-	-	1	1	1
Polyt. alpinum	1	1	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-	1	-	1	1	1	-	1
Galium saxatile	-	1	1	1	1	1	1	1	1	1	1	1	-	1	1	-	1	1	1	1	-	1	1	1	1	1	1
Nardus stricta	-	-	-	1	-	1	1	-	-	-	1	1	1	1	-	1	-	-	1	1	-	1	1	-	-	1	1
Rhytid. squarr	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1	1	-	1	-	-	-	1	-
Rhytid. loreus	1	-	1	-	1	1	1	1	1	1	1	1	1	-	1	1	1	1	1	1	1	1	1	1	1	-	-
Dryop. expansa	1	1	-	-	-	-	1	-	1	1	-	1	1	-	-	-	1	1	-	-	-	-	-	-	-	-	1
Plagioth und.	1	1	-	1	1	-	-	-	-	1	-	1	1	1	-	-	-	-	1	1	1	1	-	1	-	-	-
Viola palustris	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	1	1	1	-	1
Pellia epiphylla	-	-	-	1	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1	1	1	1
Hylocom sple.	-	-	1	-	-	1	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	1	1	-	-
A. flexile	1	-	1	-	1	-	-	1	1	1	-	1	1	-	-	1	1	1	1	1	1	1	-	-	-	-	1
Dicran. scop	1	-	-	1	-	-	-	-	1	-	-	1	1	1	1	1	1	1	-	1	1	-	-	-	1	-	-
Hypn. cupress	-	-	-	-	1	-	1	-	-	-	-	1	-	1	1	-	-	1	1	1	-	1	-	1	-	-	-
Trito. quinqu	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	1	-	-
Viola riviniana	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	
Marsup emarg	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-
Rum. acetosa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	1	-	-	-	-	-
Luzula sylvatica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-
Gymno. pheg	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thymus polytr.	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-
Alchem alpina	-	-	-	-	-	-	-	-	1	1	-	1	1	-	1	1	1	1	-	-	1	-	1	1	-	1	-
Pleuro. schreb.	-	-	-	-	-	-	-	-	-	1	1	-	-	-	1	-	-	-	1	-	-	1	-	-	1	-	-
Anthox odoratu	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	1	1	-	-	1	-	1	-	1	1	-
Camp. rotund	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Junc.squarr.	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Oreopteris lim	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1	1	1	-	-
Festuca vivip	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	1	1	-	-
Polytr comm	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-
Carex bigelow	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1
Blechnum spic	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	1	1
Anemone nem	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1

The first TWINSPAN subdivision (Table 3.6, column1) grouped the Ben Alder vegetation with the single quadrat from Beinn Eibhinn and one from Bridge of Orchy. These quadrats had larger components of *Cryptogramma crispa, Huperzia selago*, *Diplophyllum albicans*, *Kiaeria falcata* and *Hylocomium umbratum* than elsewhere. At Beinn Eibhinn, there was a particularly high proportion of rock and bryophyte cover. Together with the species in common with all three subdivisions, this association corresponds most closely to the NVC U18: the *Cryptogramma crispa-Athyrium distentifolium* snowbed. This group possibly indicates longer snow lie than the other subdivisions. McVean and Ratcliffe (1962) separated the Cryptogrammeto-Athyrietum chionophylum from tall herb vegetation, which includes ferns, through the presence of abundant *Cryptogramma* which requires late-lying snow at these altitudes. Ben Alder has large populations of *Cryptogramma crispa* on the floor of the corrie. The *Athyrium* tends to grow higher up the side of the corrie but is intermixed with *Cryptogramma*.

In Norway, Odland (1995) recorded two hundred and eleven 5 m^2 quadrats of sites with *A. distentifolium*. Of these, only eight gave a habitat very similar to the Ben Alder-Beinn Eibhinn type recognised in this first division (Table 3.6). He classified this as an alpine meadow, the Gnaphalium supinum-Salix herbacea type, characterised by *A. distentifolium* which was no taller than 50 cm and had distinctly different ground vegetation compared with all other sites. None of the other quadrats he included had species which were specific to snow beds, while this type had *Kiaeria starkei, Polytrichum sexangulare* and *P. alpinum* with *Cryptogramma crispa* and *Rumex acetosa*. The stony ground, with up to 50% rock cover contrasted with the dense vegetation cover he found elsewhere. While *Gnaphalium supinum* and *Salix herbacea* were not found in any of the first group quadrats (Table 3.6) they were observed in the general vegetation at Ben Alder, Beinn Eibhinn and Bridge of Orchy. Earlier work in Scandinavia, (Gjaerevoll, 1950), identified the Cryptogrammo-Athyrion alpestris community which describes this association.

Most of the Bridge of Orchy quadrats, together with the grass-rich quadrats from Creag Meagaidh, formed a second group (Figure 3.6, column 2). *Athyrium flexile* was more frequent, together with *Alchemilla alpina*, *Dicranum scoparium*, *Hypnum cupressiforme, Plagiomnium undulatum*, *Pleurozium schreberi, Deschampsia cespitosa*, *Luzula sylvatica*, *Gymnocarpium dryopteris, Campanula rotundifolia* and *Rumex acetosa* which distinguished this group. *Cryptogramma crispa* was present but less frequent. There was more *Dryopteris expansa* and *Gymnocarpium phegopteris* than had been observed elsewhere. Ferns were the major component of the vegetation. This association approaches the U16 *Luzula sylvatica-Vaccinium myrtillus* tall-herb community in the U16a classification; the *Dryopteris dilatata-Dicranum majus* sub-community, but it still has close affinities with U18 and many of the species overlap. There is a significant proportion of *Deschampsia caespitosa* which was most marked in two of the Creag Meagaidh quadrats, one of which had up to 50% cover. McVean and Ratcliffe (1962) linked the presence of this species to high grazing levels and suggested it replaced the tall herb association. They made a comparison with extensive ungrazed areas of cliff, as at Beinn Bhan in Applecross and Caenlochan in Angus, where the grazed areas have more *Deschampsia*.

Unstable screes, which certainly prevail in the fern-rich areas at Bridge of Orchy, have helped to ensure the survival of these ferns. McVean and Ratcliffe (1962) listed *Geranium sylvaticum* and *Trollius europaeus,* for example, which occur abundantly on the cliffs above the ferns at Bridge of Orchy, but are only occasionally seen as small flowerless plants in the corrie below. Part of the backwall of the corrie is basic metamorphic rock and this brings enrichment through seepage, providing the nutrients for potentially taller vegetation than sites like Ben Alder. With less grazing, this site might be a fern meadow with a greater range of species. Odland (1995) described two types of meadow where *A. distentifolium* grew up to 1.5 m, both with little ground vegetation due to the dense canopy of ferns. These approximate to the herb-rich meadows of McVean and Ratcliffe (1962). Odland listed *Cicerbita alpina* as a typical species of the tall herb meadows in Norway, but it is only found very rarely on cliff ledges in Scotland, where *A. distentifolium* also grows. These

meadows were included in the Scandinavian Lactucion alpinae association (Gjaerevoll, 1950) which contrasted with the Cryptogrammo-Athyrion alpestris with shorter vegetation. A third type of meadow in Norway may not emerge from the snow until August, but such late emergence is not found in Scotland (McVean and Ratcliffe, 1962).

In the third division (Table 3.6, column 3), three of the quadrats from the margin of the scree at Bridge of Orchy were grouped with two from Glen Prosen to give an association particularly marked by *Viola palustris, Blechnum spicant, Oreopteris limbosperma, Anemone nemorosa*, *Pellia epiphylla* and *Festuca vivipara*. This association was again similar to U18, but perhaps distinguished areas which were among the earliest to emerge from the snow, but also consistently moist. Examining all the species present, this association suggests McVean and Ratcliffe's (1962) Herb-rich birchwood. This illustrates the presence of species which indicate former woodland. These particular quadrats are in are the lower-altitude sites, and the occasional cliff-bound *Sorbus* and the *Salix lapponum* at Bridge of Orchy and Glen Prosen indicate at least the proximity of woodland scrub. Odland (1995) recognised a distinction between higher altitude sites and those which extended to lower altitudes, with more tree cover. He divided the lower altitude sites into three types, none of which directly correspond to habitats in this country. Odland gave *Luzula sylvatica* and *Blechnum spicant* as examples of species which illustrate the oceanic influence, which is appropriate for Scotland, and *Luzula* was noted at all the sites. Different associations for *A. distentifolium* within woodland are described in Scandinavian, European and North American sites (Davis, 1965; Il'in, 1968; Cody and Britten, 1989), but such divisions cannot be made in Scotland, other than from relict vegetation. Several of Odland's communities were among birch and a variety of willows. He also mentions the occurrence of *A. distentifolium* in its lowest sites among *Quercus* and *Fagus* (Odland, 1995). In Europe, *Alnus viridus* communities offer a suitable habitat and *Picea abies* also offer a very different association to the typical environment with which *A. distentifolium* is associated in Scotland (Odland, 1995).

While the Scandinavian communities do have some similarities, Odland (1995) stated that the majority of the *A. distentifolium* stands are found among the Lactucion alpinae alliance in an association which he estimated to be one of the least anthropogenically disturbed habitats. As the nearest approximation to this alpine meadow in Scotland is confined by grazing to cliff ledges, we have a greatly reduced representation of this type of vegetation. But many of the rocky, acidic Scottish sites with low vegetation offer a very specific environment on a scale which might not be found elsewhere.

Several fungi were found on decaying fronds of *A. distentifolium*. *Mycena cinerella* Karsten was found abundantly at Bridge of Orchy in October 1995-97 and Glen Prosen 1997. *Mycena metata* (Fr: Fr) Kummer was found on decaying frond bases at Fuar Tholl, Wester Ross, in August 1997. *Galerina calyptrata* Orton was found on *A. distentifolium* litter in the middle of the field site at Glen Prosen in July 1997. *Ramariopsis subtilis* (Pers. Fr) Corner *s. lato*, was found at Bridge of Orchy, in October 1997. All are not uncommon, with no alpine affinities, and are found in broad-leaved woodland (Courtecuisse and Duhem, 1995).

(Identification by Adrian Newton, Rosemary Smith, Mary Clarkson and Roy Watling).

3.3.2: Population sizes

A. distentifolium is found in populations varying from a few plants to thousands. The *A. flexile* populations are generally much smaller. Distinct clumps might be assumed to be the same clone (see 4.8) but it is difficult to assess how many individual plants are present among well-dispersed crowns. At Glen Prosen there were 96 crowns of *A. flexile* around the main site. With gentle probing, many of these were found to belong to the same clone. There were also two clumps of *A. flexile* further down the slope. At Ben Alder there were several hundred clumps of *A. flexile*, as at Beinn Eibhinn and the main corrie at Bridge of Orchy. Only eight individual clumps were found in the other site at Bridge of Orchy. Four clumps were found at Creag Meagaidh. In Glen Einich, there was only one large clump and a single crown one metre away.

3.4: Response to adversity

3.4.1: Grazing

Fifty plants of each taxon at Ben Alder were scored in a traverse across the corrie to assess the percentage of whole clumps which had been eaten (Table 3.7). Separate populations were identified at intervals of 30-200 m and the first five clumps to be encountered were scored. Grazing was very variable depending on the terrain. Accessible plants among stable rocks were often grazed; those among large unstable boulders were not. Of the 20-30% which had been grazed, up to 90% of the foliage had been removed (Table 3.7).

Table 3.7 Visual estimate of the % of individual plants grazed at Ben Alder on 8th July 1997

50 clumps of each	% clumps with some damage	% frond area removed from grazed plants	SE	Range of biomass removed
A. flexile	30%	49%	7.27	5-90%
A. distentifolium	20%	44.2%	8.78	1-70%

This might represent a significant impact over a period of time. These plants would be obliged to use their reserves to produce new fronds and this could help to explain why so many are infertile. Red deer are the only large grazing animals at Ben Alder, with the possible exception of mountain hares.

A different method of scoring was used on the marked plants at the field sites, as individual fronds were scored. At Glen Prosen in 1996 the *A. flexile* plants beneath the rocks were relatively protected and were only nipped at the tips. Five of the larger clumps of *A. distentifolium* in the centre of the rocks had many of their fronds almost entirely removed in 1996, and this had also been observed over most of the clumps in 1995. In 1997 both taxa produced slightly fewer mature fronds as many were frosted and had to be replaced, but grazing was light (Table 3.8). Red deer are present in large numbers in Glen Prosen together with sheep and mountain hare. Mountain hare droppings were found at the Prosen rocks and the hares were presumably the only herbivores on fronds under overhanging rocks.

Table 3.8 Damaged fronds on monitored plants at Glen Prosen recorded in July

Year	Taxon	Total fronds	Number damaged	% fronds damaged
1996	*A. flexile*	66	4	6%
1996	*A. distentifolium*	64	24	37.5%
1997	*A. flexile*	61	5	8%
1997	*A. distentifolium*	59	4	7%

At Bridge of Orchy both sheep and deer grazed in 1996. Due to a change of policy there were mainly deer with only a few stray sheep in 1997. Grazing was not a problem either year but there was even less once the sheep had been removed (Table 3.9).

Table 3.9 Damaged fronds on monitored plants at Bridge of Orchy recorded in July

Year	Taxon	Total fronds	Number damaged	% fronds damaged
1996	*A. flexile*	88	5	6%
1996	*A. distentifolium*	92	5	5%
1997	*A. flexile*	81	1	1.2%
1997	*A. distentifolium*	99	0	0%

3.4.2: Competition

Plants of snowbed communities avoid competition from many other plants by occupying a specialised habitat. The dead fronds are slow to decay and form dense litter layers. *Pteridium aquilinum* was found to release toxins from the roots and rhizomes, which were emitted as temperatures rose after the winter (Gleissman, 1976). This coincided with renewed growth in other plants. Munther and Fairbrother (1980) found that fern fronds emit a toxic compound which can be leached out of the fronds by rain. The leachate from *A. distentifolium* might have similar effects. In a dense population this could also inhibit the growth of gametophytes near the parents and helps to explain the lack of young plants in large established colonies.

3.4.3: Freezing

As arctic-alpine taxa, it might be expected that *A. distentifolium* and *A. flexile* have particular adaptations to withstand low temperatures. This is not necessarily so. The lowest temperature the Bridge of Orchy thermometer reached overwinter was -4 °C which implied good snowcover during periods of lower temperature, even though there was less snow than usual. One consequence of extended snow cover is an increase in CO_2. Woolgrove and Woodin (1996a) found CO_2 levels up to 70 µl/l above the ambient level. Not all plants can tolerate this. *Athyrium filix-femina* can occur at these higher altitudes and Odland (1981) suggested that *A. filix-femina* occurred only where it was not covered by long snow lie. Possibly, this implies a difference in tolerance of CO_2 levels and marks a specific adaptation by *A. distentifolium* to snowbed conditions.

Sato and Saki (1981b) found that senescent *A. distentifolium* sporophytes could withstand freezing to -15 °C for one day but were killed at -20 °C. A small experiment was conducted with plants grown from spores collected at the field sites. Trays of one year-old sporophytes containing six *A. distentifolium* and six *A. flexile* were grown in the greenhouse at 20 °C for twelve weeks. In the autumn, they were placed in an open cold frame for eight weeks, and eventually experienced light frost at -1 °C. They were taken straight from outside and were placed into freezers (with no gradual acclimatisation) at -6, -10 and -20 °C for one day, one week and four weeks. Three *A. flexile* plants and one *A. distentifolium* survived at -6 °C for one day, only one *A. flexile* at -6 °C for one week and no other sporophytes survived being frozen (Table 3.10).

Table 3.10 Recovery of sporophytes frozen at a range of temperatures
F = *A. flexile*. *D* = *A. distentifolium*.

	-6 °C		-10 °C		-20 °C	
	F	*D*	*F*	*D*	*F*	*D*
1 day	3	1	0	0	0	0
1 week	1	0	0	0	0	0
4 weeks	0	0	0	0	0	0

These plants were frozen solid which would probably not have happened in the wild. Also the temperature would probably have changed more gradually. Large sporophytes in pots survived overwinter 1994-95 in a cold frame that experienced temperatures as low as -7 °C.

Gametophytes were treated in exactly the same way, and given the same chilling in the cold frame, except they retained the cling film across the top of the pots. The results were inconsistent. Gametophytes of *A. distentifolium* from Bridge of Orchy regenerated after one day and one week at -6 °C. Ben Alder *A. flexile* gametophytes regenerated after one day at -20 °C and four weeks at -10 °C. One gametophyte in a set of *A. flexile* gametophytes from Bridge of Orchy regenerated after four weeks at -20 °C (Table 3.11).

Table 3.11 Recovery of gametophytes frozen at a range of temperatures
F = A. flexile. D = A. distentifolium.

	-6 °C		-10 °C		-20 °C	
	F	*D*	*F*	*D*	*F*	*D*
1 day	0	1	0	0	1	0
1 week	0	1	0	0	0	0
4 weeks	0	0	1	0	1	0

Sato and Saki (1981) found some marginal cells on the wings of some gametophytes were still alive after freezing at -70 °C and that a gametophyte could regenerate from a single cell. The -10 °C and -20 °C examples could have come from a thickened part of the thallus, which seemed to occur quite frequently in cultivation. This behaviour of the thallus could be further investigated.

3.4.4: Desiccation and high temperatures

One distinguishing feature of the *Athyrium distentifolium-Cryptogramma crispa* snowbed is the continuing availability of seeping moisture. The lack of adequate overwinter snow, as in 1996-97, or an exceptionally dry summer as in 1995, resulted in plants which suffered from lack of water.

Four sets of gametophytes and four boxes of senescent sporophytes of both taxa were prepared as for the freezing experiments and allowed to dry out with no water added for periods from four to sixteen weeks. They were kept in a cold store at 4 °C from 24.2.1997, so that they were not encouraged to grow. After the period of desiccation they were returned to the cold frame and well watered. The gametophytes all died, even after only four weeks. The sets of six sporophytes recovered well after four and eight weeks, but only three *A. distentifolium* and two *A. flexile* sporophytes recovered after twelve weeks, and one *A. distentifolium* after sixteen weeks (Table 3.12). This suggested that the sporophytes can withstand periods of drought better than the gametophytes, but only for a few months.

Table 3.12 Recovery of sporophytes and gametophytes given no water for up to 16 weeks. *F* = *A. flexile*. *D* = *A. distentifolium*.

	Gametophytes		Sporophytes	
	F	*D*	*F*	*D*
4 weeks	0	0	6	6
8 weeks	0	0	6	6
12 weeks	0	0	2	3
16 weeks	0	0	0	1

Gametophytes in the temperature gradient bar (5.2 & 5.3) were subjected to high temperatures for 48 hours when a thermostat was accidentally turned up. The temperature at the 25 °C point on the gradient rose to 35 °C. Of the eight dishes of gametophytes, all but three were killed. These surviving three, Glen Prosen *A. flexile*, Glen Doll *A. distentifolium* and Ben Alder *A. distentifolium* were badly browned but produced new prothallial growth from the few cells which had survived. The normal growth habit at 25 °C was a dense mass of branched prothalli, like a cushion, and this might be a useful adaptation, being less vulnerable than a large heart-shaped prothallus. On the same occasion, the gametophytes normally maintained around 20 °C experienced 29.2 °C and seemed unaffected. This suggested that extreme temperatures could be briefly tolerated in the wild and demonstrated the ability to regenerate from a few surviving cells.

3.5: Discussion and conclusions

Several factors have been seen to characterise the location of populations of *A. distentifolium* and *A. flexile*. Both taxa are usually found on north-facing slopes at the altitude and topography which receives the optimum amount of snow cover. Too little, and plants would be frozen solid or emerge to grow too soon in the spring. Too long a snow cover would bring a very short growing season. Their location against the foot of cliffs and among large boulders, can help in the eventual melting of the snow as the rock warms up and emits heat. During the winter, the sun does not penetrate into the corries and any lying snow would stay for a long time. Very late lying snow in June and July would receive direct sun, and by that time would melt more effectively through being so soiled. Glen Prosen is unusual as a south-facing

site, although most of the smaller ferns are partially shaded below the rocks. The site appears to be especially vulnerable to frost damage. At Bridge of Orchy the summer sun does not shine onto the ferns until the afternoon, which would cause less damage from a light air frost on foliage. The rocks can become very warm, but the depth of the scree would help to insulate against excessive heat. In a normal season there is seepage of water from above, either from melting snowbeds or groundwater. This combination makes for rapid expansion of the plants.

Each site is a different combination of rock type, aspect, altitude and grazing regime. All are found in areas of late-lying snow, but even in these few sites which have been examined in some detail there is an indication of varying vegetation types. In most sites, the species composition has been modified by grazing, and some of the present vegetation might reflect remnants of former woodland. Fern meadows occur among less stable screes or on inaccessible ledges, when this type of vegetation might formerly have been more widespread.

As the populations of *A. distentifolium* in Norway are geographically nearest to Scotland, it is tempting to draw parallels. However the Scottish climate, with a mixture of oceanic and alpine weather patterns, is different from either the Norwegian or the European climates. While there are similarities in the vegetation there are also marked differences which give Scottish vegetation a distinctive character. Odland (1995) found pH values ranging from 3.8 to 5.5, and lower values were unusual. The sites where *A. flexile* and *A. distentifolium* were found together had pH 3.5-4.5. The appearance of snowbed species was unusual in stands of Norwegian *A. distentifolium*, while that is the more usual habitat for Scottish *A. distentifolium*. In Norway, *A. distentifolium* can be found as low as 300 m a.s.l. with prolonged snow lie. In Scotland, the snow would not lie so long at such a low altitude.

All the sites of *A. flexile* that have been located are restricted to the Central Highlands. *A. distentifolium* does grow in the North-west Highlands, but except for

the doubtful record from Ladhar Bheinn on Knoydart, *A. flexile* has not been recorded. Similar climatic conditions occur in both areas, but there is a difference in the number of days with lying snow (Figure 3.5) and this might prove to be an important factor in determining where this taxon can be found.

The montane habitat has changed and has been considerably reduced and fragmented by climatic changes. In additional to natural processes, anthropogenic effects could have a significant influence. While exceptional seasons have always occurred, the possibility of global warming adds another dimension. In addition, specific pollutants could be accentuating these montane communities' response to warming. Many areas of Scotland have become naturally more acidic over the last ten thousand years. Some soils can naturally replenish their mineral content as rocks decompose, but in granite areas, decomposition of the rock to provide minerals is slower than the effect of leaching. These are the areas which are most affected by the additional acidity of acid rain as the soils and peat are especially vulnerable. Wilson *et al.* (1989) estimated that more than 25% of the soils in Scotland have a low pH, but have become even more acidic through addition from acid rain. A further 36% which were not naturally so acidic are in danger of becoming more so and cannot neutralise the additional acidity. Fractionation of the ions within a snowbed has been found to concentrate the acidic pollutants in the lower layers. Fifty to eighty % of the ion load is released in the first 20% of snowmelt to give an acid flush and bryophytes like *Kiaeria starkei*, which occurs in *A. distentifolium* snowbed vegetation have been damaged (Woolgrove and Woodin, 1996b). Meltwaters have been found to contain high levels of nitrate and sulphate resulting in a pH as low as 3.2 (Lee *et al*, 1989). Acidification can lead to slower growth. Increasing sulphur dioxide levels in the Netherlands were correlated with a decline in the abundance of pteridophytes like *Lycopodium clavatum* and *Polypodium vulgare* (Lee *et al*, 1989). Field measurements (5.5) have shown that there is usually a time-lag of several weeks from first emergence from the snow until first growth. This could protect the new young fronds from damage to the leaves which bryophytes incurred, but there is still

the possibility of the soil becoming increasingly acidified, affecting the roots and nutrient cycling.

At Corrie Dhondail in the Cairngorms in 1997, there were many plants which were browned and had produced new growth in July. Beinn Eibhinn plants in September 1996 were also brown. They may have been indicating more than a dry spring or an early frost. Pollution in snow, cloud and rain has particularly detrimental effects on high altitude plants. Hill cloud forms where rising air, which may be polluted, cools at the condensation level. Within these clouds, water condenses around pollutant particles. With increasing wind speed and droplet size these pollutants are transferred to plant surfaces. Rain falling through the hill cloud also causes precipitation of the concentrated pollutants (Grace and Unsworth, 1988). This gives a greater acid deposition in upland rain as the higher precipitation in montane areas gives a increased input of pollutants. The prevailing winds determined the area most affected by air pollution. Westerly winds from the Atlantic Ocean bring clean air, while winds from the continent contain impurities (Fowler and Irwin, 1989). Generally there is cleaner air in the north and west, which receive relatively little pollution compared with the rest of Europe. This coincides with much of the present distribution of *A. distentifolium*, but pollutants can be concentrated within snow beds as described above.

Nitrogen deposition in the Southern Uplands and South-west Scotland is 25-50 kg N ha^{-1} yr^{-1}compared with 5-10 kg N ha^{-1} yr^{-1} in lowland Scotland (Cannell *et al*, 1997) and this illustrates the higher deposition on high ground. As many less common slower-growing species occur in sites which are nitrogen deficient, this advantage is lost if this niche is enriched and other more invasive species can grow. Additional nitrogen could also cause a flush of premature growth and make the plants more vulnerable to frost damage (Lee *et al*, 1989). Plants are progressively weakened if they have to produce extra growth after a mild spring with late frosts, as happened at Glen Prosen in 1997.

Ozone concentrations have doubled throughout the 20th Century and the level is still increasing. Levels above the calculated threshold of 40 ppb occur in the Highlands throughout the growing season of April to September. While the precise effects on natural vegetation are not known, experiments on crop plants have demonstrated that these levels were more damaging than sulphur or nitrogen deposition (Cannell *et al*, 1997). The combined effect of all these pollutants, together with global warming, could change the habitat and destroy the snowbed communities. Other species would be able to grow at higher altitudes and the competitive advantage of the specialised snowbed environment might be lost.

CHAPTER FOUR Morphology

4.1: Introduction

The information in this chapter was gathered to make comparisons between, and descriptions of, the morphology of both taxa. A morphometric analysis was designed to compare the whole frond of *A. flexile* with *A. distentifolium* plants from within Scotland and abroad. Arising from this series of measurements, further analysis compared the height of fronds from different sites and countries. More detailed microscopic features were examined to look for differences in the number of cells in the annulus, the spores and stomata. The chromosome numbers were confirmed, as these previously had only once been counted on small samples. Rhizomes were measured and compared, to look for differences between sites and taxa. Some were excavated to reveal their extensive development. Samples of roots were monitored at the field sites over two field seasons to provide an indication of mycorrhizal activity. Mycorrhizas could make a significant contribution to the nutrition of these plants in the low-nutrient habitat which characterises most of the populations of *A. flexile* and *A. distentifolium*.

4.2: Morphometric analysis

4.2.1: Introduction

Morphometric analysis is a technique that uses a series of measurements that are analysed by computer to suggest similarities or differences between a range of individuals. Pryer *et al*, (1995) for example, used this technique for 77 characters on samples from 50 taxa to independently assess the phylogenetic relationships between the taxa. Features chosen to distinguish between *A. flexile* and *A. distentifolium* covered a narrower range of variation than Pryer's study, but some of the characters measured were similar. When *A. flexile* was first discovered, it was named as a species because it looked significantly different from *A. distentifolium*. To test whether these morphological characters are consistent, fourteen taxonomic features were chosen from descriptions of the two taxa (Newman, 1853; Page, 1982).

Emphasis was laid on features which represented opposite extremes. After assembling a detailed series of measurements, a multivariate analysis of these data, gave the opportunity to establish whether two clear groups would emerge.

4.2.2: Materials and methods

The fronds which were measured were a combination of herbarium material from the Royal Botanic Garden Edinburgh and specimens collected from field sites. No cultivated material was used. Fronds from the monitored plants were measured for two years in succession. In all, 100 *A. flexile* were measured, and 144 *A. distentifolium*. Some of the *A. distentifolium* in the herbarium were non-Scottish and additional examples were obtained from Oslo and Vienna. More than three hundred Scandinavian specimens were examined and thirty were measured. Ten specimens from Vienna were fertile and also included in the sample. Some of the earlier specimens to be measured were not fertile and this gave incomplete data so that later specimens were selected for mature sori. The sources of the fronds are given in Appendix 1a.

Fourteen characters were measured. *A. flexile* has a narrow frond throughout most of its length while *A. distentifolium* is broader in the middle of the blade and tapers more towards either end. This was measured by a ratio based on the length divided by the width (1). A similar ratio was derived for the comparatively shorter stipe of *A. flexile* and the longer one of *A. distentifolium* with a ratio comparing the length of the blade with the length of the stipe (2). *A. flexile* has the broadest part of the blade somewhere between the base of the frond and midway; *A. distentifolium* is usually broadest around the middle of the blade. A ratio was constructed from the length of the blade and the distance to the widest point from the base of the blade (3). Another ratio was obtained from the length and width of the pinnules (4), as *A. flexile* appeared to have shorter, broader pinnules. *A. distentifolium* has well-spaced lower pinnae and the number of pinnae in the lowest quarter gave a comparison with *A. flexile* (5). The deflexed lower pinnae of *A. flexile* suggested measuring the angle between the rachis and the pinnae (6) as *A. distentifolium* pinnae are usually at right

angles to the rachis at the mid-point in the blade. The terminal veins in the pinnules forked and some of the pinnules had very blunt tips with two or more teeth. This gave two areas to score: the forked veins (7) and the closely related number of level points at the tip of each pinnule (8). The number of sinuses (9) (indentations between the teeth) on each pinnule were counted, as *A. distentifolium* tended to have longer, more divided pinnules. When individual pinnae were measured, they were taken at the widest point of the blade. *A. flexile* appeared to be more scaly and this was assessed by counting all the scales on the rachis beyond the broadest point (10). A percentage was given for how much of the frond was fertile (11), and a numbering system indicated where the sori were located. Fronds fertile at the base scored 1, the lower half 2, lower three quarters 3, whole frond 4. The upper quarter scored 5, upper half 6 and upper three quarters 7 (12). The number of sori were counted on the pinnules nearest the widest point, or the nearest fertile pinnules to that point (13). Finally, a microscope was used (x 100) to count the number of cells in the annulus, the thick walled line of cells on the sporangium which control spore release (14).

The SYNTAX multivariate analysis program (Podani, 1993) was used to construct a principal components analysis. The measurements were expressed as ratios so that the size of the fronds would not influence the results. The ratios were converted to Log 10, as were the mean numbers of sinuses, broad scales, sori and cells in the annulus. The other values were less variable and were not converted. Missing values, of infertile, or occasional fronds collected without a full stipe, were excluded from the analysis using the SYNTAX missing-value procedure.

4.2.3: Results

The eigenvalues of the principal components analysis showed that the first two axes explain 40% of the information (Table 4.2.1).

Table 4.2.1 Variable loadings on the first four axes with percent eigenvalues for each axis. The variables showing the highest correlations with each axis are shown in bold.

Variable	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues (%)	*28.2*	*12.8*	*7.6*	*7.3*
1. blade/stipe ratio	**0.5119**	0.0838	-0.3187	-0.2971
2. blade/width ratio	0.2537	0.3390	**-0.5764**	0.3944
3. distance to maximum width of blade	0.3587	-0.2293	**0.4174**	**0.4647**
4. length/width pinnule ratio	**-0.5228**	0.3519	0.2399	-0.0609
5. number of pinnae in lower quarter	**0.4414**	**0.5883**	0.0058	0.1302
6. angle of pinnae at mid-point	0.3452	0.0640	**0.3714**	0.0662
7. number of level points of pinnule	**0.7159**	0.0884	-0.0151	-0.3090
8. number of sinuses on pinnule	**-0.8500**	-0.0375	0.0634	-0.0540
9. forked veins at tip of pinnule	**0.5215**	-0.0736	0.1976	**-0.5332**
10. number of broad scales	0.2623	**0.6679**	**0.4402**	0.0354
11. proportion of blade fertile	-0.3707	**0.5779**	0.0121	0.0435
12. position of fertility	**-0.6657**	-0.1629	0.0747	-0.2386
13. number of sori	**-0.8311**	0.1728	-0.0341	-0.0015
14. number of cells in the annulus	-0.2391	**0.5318**	-0.1099	-0.3065

When the first two axes are plotted as a two-dimensional graph, two clear groups are seen with some intermediate examples (Figure 4.2.1). Fifteen *A. flexile* and thirty-four *A. distentifolium* fronds are not shown as they are directly underneath other points. When the two groups are plotted separately on the same axes, the general distribution is much the same, indicating that the points have plotted directly over another similar example from the same taxon and useful data are not obscured. Individual fronds may be identified by number, but the resulting plot is very crowded and difficult to interpret (Appendix 1b). The four *A. flexile* data points high on axis 1, come from Glen Prosen and were sterile, as were the two *A. distentifolium* also shown in that region.

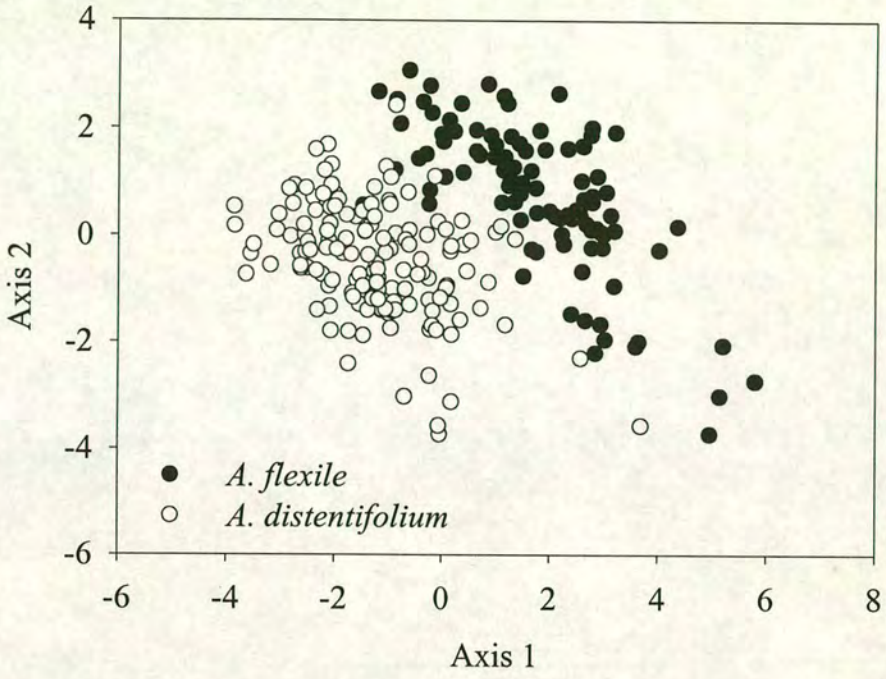


Figure 4.2.1 A principal components analysis of 100 fronds of *A. flexile* and 144 fronds of *A. distentifolium* plotted on the first two axes showing two main groups with some intermediates.

When the loadings of the fourteen variables are superimposed onto the principal components (Figure 4.2.2) some are clearly associated with the axis separating the two taxa. This indicates which variables are best described for discriminating between the taxa. Of the fourteen variables, *A. distentifolium* particularly has high values of Variables 4, 8 and 13 represented by longer pinnules with more sinuses and sori (Table 4.2.1). The position of fertility is very different for *A. distentifolium*, being predominantly terminal, indicated by Variable 12. *A. flexile* is the opposite of *A. distentifolium* with shorter pinnules, fewer sinuses and sori and a different position for the sporangia on the frond. *A. flexile* also has high values of Variables 1, 2 and 3 that indicated the blade is a markedly different shape. Variables 5 and 6 illustrate the difference in the number of pinnae near the base of the frond, as there are relatively more in *A. flexile*, and the pinnules have more forked veins and level points; Variables 7 and 9. Variable 10 showed that there are more scales continuing onto the

upper part of the rachis (Table 4.2.1). Variable 11, the proportion of the blade fertile, was not associated with either group and only early examples were used which were not fertile. It indicates that some outlying fronds of both taxa owe their position in the ordination to infertility more than any other character. This character was less useful than others. The number of cells in the annulus, Variable 14, also was not strongly associated with the discriminating axis, but will be further discussed in Section 4.4.

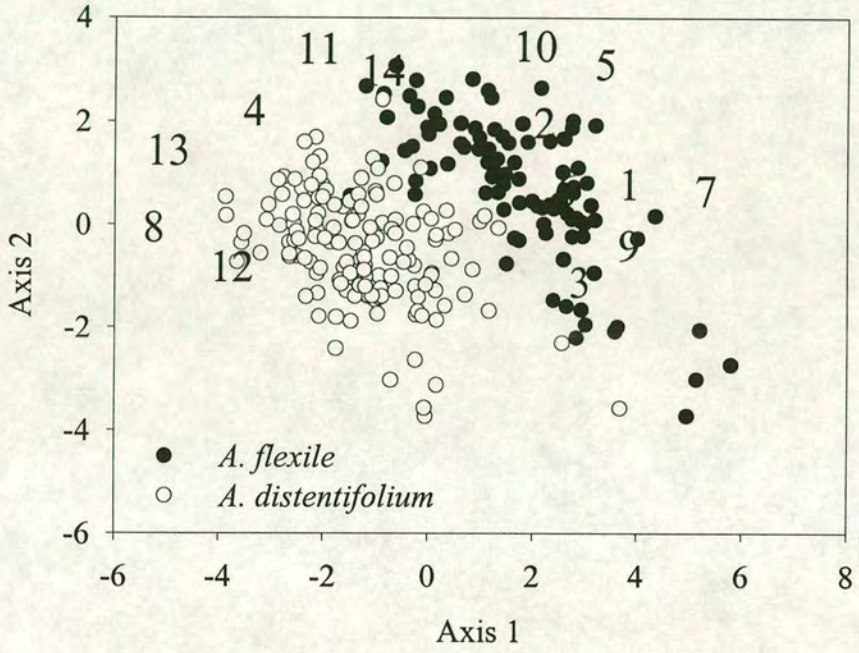


Figure 4.2.2 Biplot with the loadings for the 14 variables superimposed on the first two principal components showing that 12, 8, 13 and 4 are stronger characters for *A. distentifolium* and 10, 5, 2, 6, 1, 7, 9 and 3 best describe *A. flexile.* 11 marks the more fertile fronds with the infertile ones of both taxa high on Axis 1. Variable numbers are as in Table 4.2.1.

Although there were fewer specimens of *A. flexile* than *A. distentifolium*, 100 compared with 144, the *A. distentifolium* fronds plotted closer together.

When the Scottish *A. distentifolium* is identified separately from the plants from other sources (Figure 4.2.3), Scottish *A. distentifolium* is intermediate between the *A. flexile* fronds and the *A. distentifolium* from elsewhere. Without the Scottish *A. distentifolium* there would be a wider separation between the two groups.

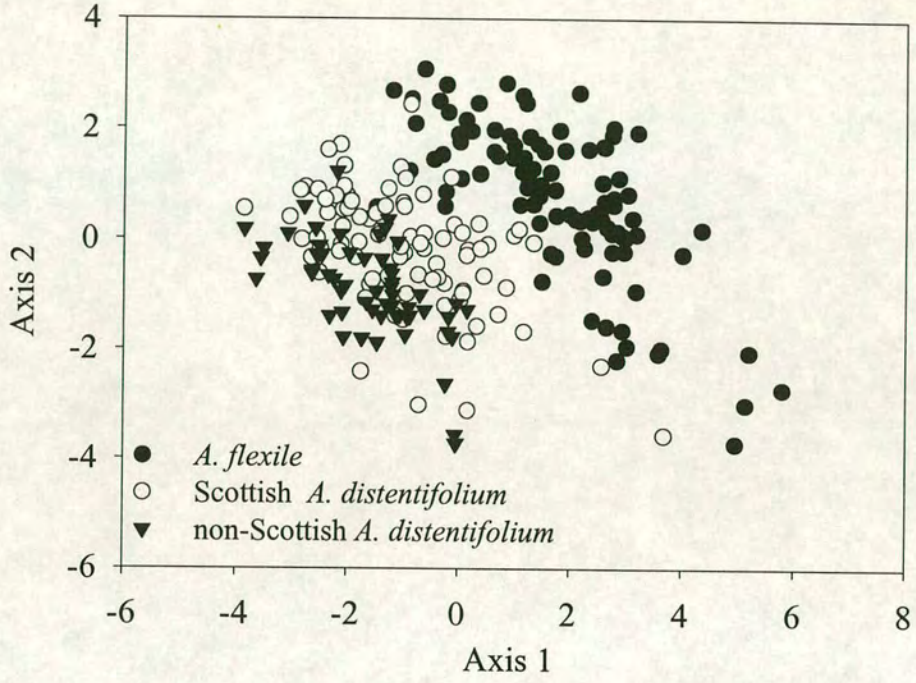


Figure 4.2.3 The first two principal components of morphological data with *A. distentifolium* differentiated into Scottish material and plants from elsewhere. Scottish *A. distentifolium* is morphologically intermediate between *A. flexile* and *A. distentifolium* from other parts of the northern hemisphere.

When only the *A. distentifolium* is plotted there is little differentiation between the fronds from different sources outside Scotland. In a plot of non-Scottish *A. distentifolium* (Figure 4.2.4) there are six fronds from North America which plot reasonably close together and these are morphologically distinct with longer, deeply cut pinnules but they have plotted well within the rest of the *A. distentifolium*. The thirty-eight Scandinavian fronds are intermixed with fourteen from Central Europe.

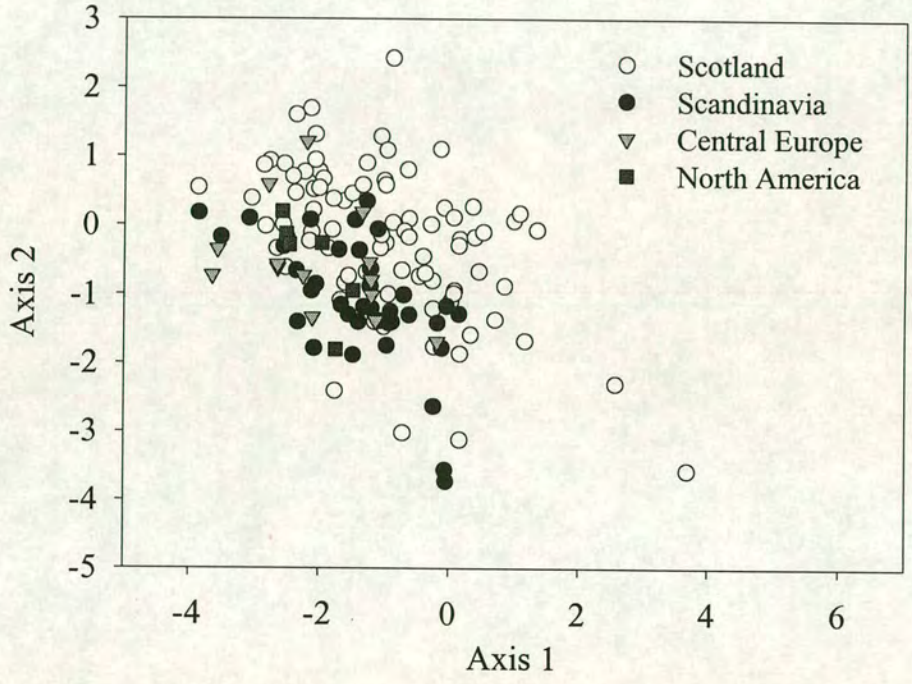


Figure 4.2.4 Principal components analysis of morphometric data for both *A. distentifolium* and *A. flexile* (*A. flexile* not shown), giving the different provinces of origin of *A. distentifolium*. The fronds from Scotland are more on one side of the cluster while intermixed with those from Scandinavia, Europe and North America.

The Scottish *A. distentifolium* (Figure 4.2.5) show little internal partitioning except for the plants from Bridge of Orchy that congregate high on the second axis. This might be associated with high fertility; Variable 11.

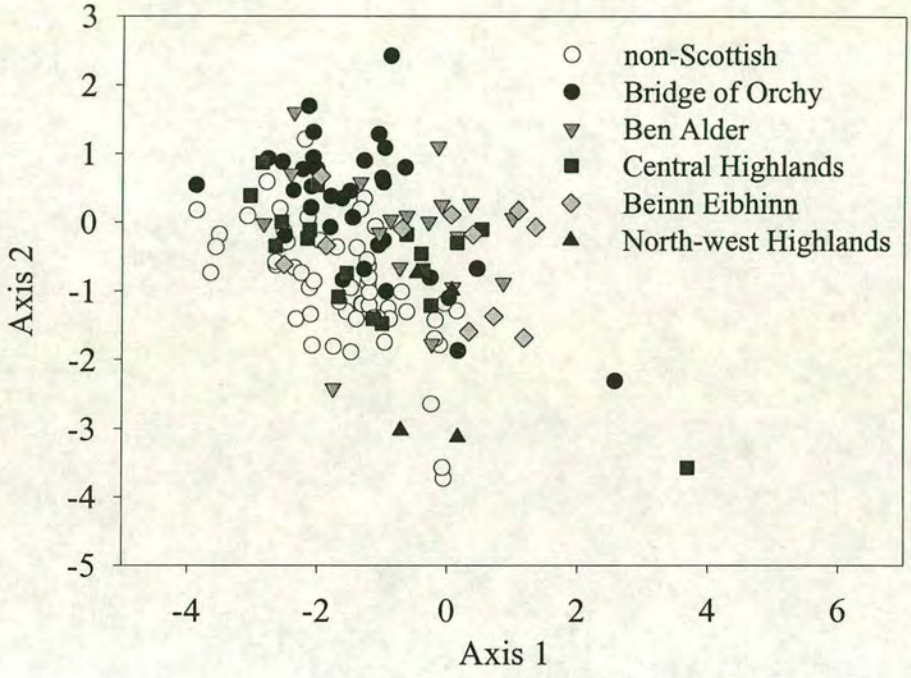


Figure 4.2.5 Principal components analysis of morphometric data for both *A. distentifolium* and *A. flexile* (*A. flexile* not shown), showing the sources of Scottish *A. distentifolium* with the non-Scottish fronds undifferentiated. The Scottish populations are intermixed around the centre of the cluster but the Bridge of Orchy plants tend to be higher on the second axis.

A plot showing only the *A. flexile* fronds (Figure 4.2.6) illustrates the repeated pattern of the Bridge of Orchy fronds plotting high on the second axis. The geographically closer Ben Alder, Ben Eibhinn and Creag Meagaidh are grouped nearer the centre of the distribution intermixed with the Glen Prosen fronds that extend the plot high on the first axis. Variable 14 (Figure 4.2.2), the number of cells in the annulus, is associated with the upper end of this linear range. The Bridge of Orchy *A. flexile* generally had higher fertility but a lower numbers of annulus cells than many of the plants from other sites.

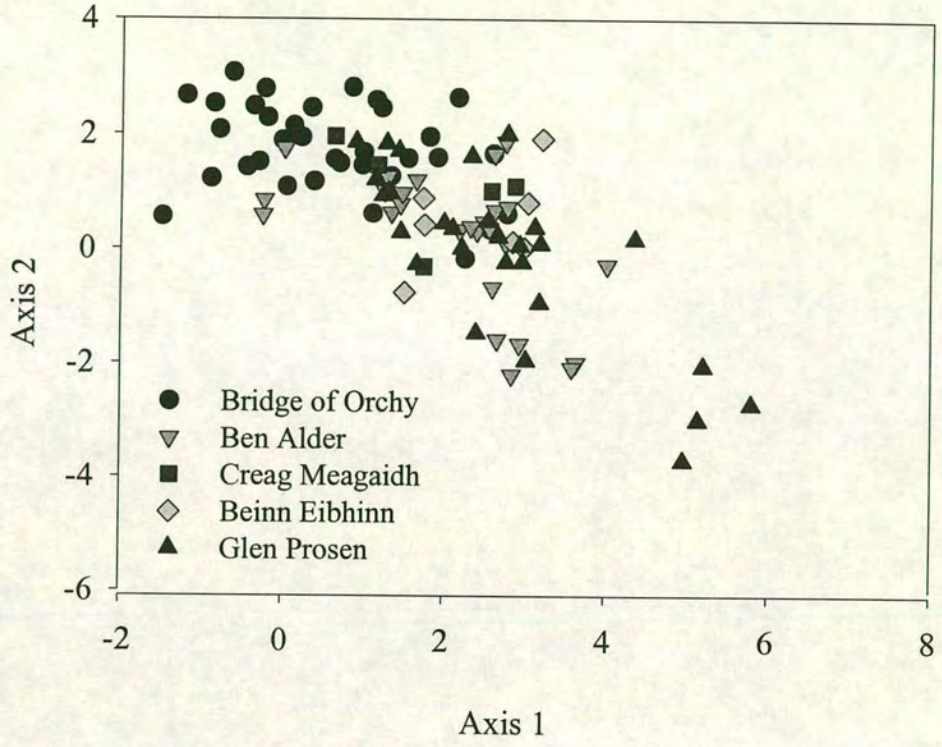


Figure 4.2.6 Principal components analysis of morphometric data for both *A. distentifolium* and *A. flexile* (*A. distentifolium* not shown) with the sources of *A. flexile* fronds, showing the plants from Creag Meagaidh, Beinn Eibhinn and Ben Alder towards the centre of the cluster, with the Bridge of Orchy fronds plotting high on the second axis and the Glen Prosen fronds high on the first axis.

4.2.4: Discussion and conclusions

These results indicate that it is generally possible to separate *A. flexile* fronds from *A. distentifolium.* While some fronds from either taxon link the two clusters, there is a segregation into two areas which are most densely occupied (Figure 4.2.1). When the *A. distentifolium* is identified as Scottish or non-Scottish (Figure 4.2.3), the representatives of *A. distentifolium* from elsewhere in the world are seen to be morphologically more different from *A. flexile* than the Scottish *A. distentifolium.*

Despite attempting to remove the size factor, the number of sinuses and large number of sori are related to the larger pinnae *A. distentifolium* has to accommodate these (Figure 4.2.2). The position of fertility, Variable 12, is the one variable most clearly identified with *A. distentifolium*, and thus in the opposite application is relevant to *A. flexile* and the basal fertility is a distinct factor in identifying *A. flexile* fronds in the field.

The variables were selected to show a difference, and this has been successful for the *A. flexile*. The American *A. distentifolium* which is morphologically distinct, has plotted reasonably close together (Figure 4.2.4), but still within the range of other *A. distentifolium* and is not sufficiently distinct to form a morphologically separate group even though it has been called *A. distentifolium* subsp. *americanum* (Cody and Britten, 1989). Even when plotted on the third and fourth axes, it was not possible to obtain a clear separation.

Just over one quarter of the samples of both taxa came from Bridge of Orchy as this was a prolific site and frequently visited. The fronds are conspicuous in the plots, but instead of being randomly distributed through the general distribution, they stand out as being more fertile and morphologically distinct. This is also true in the field. The *A. flexile* plants from Ben Alder, Ben Eibhinn, Creag Meagaidh, Glen Einich and Glen Prosen are very similar. They are usually comparatively small, and have the characteristic bend near the base of the stipe. The plants from Bridge of Orchy are larger, sometimes very broad, but still with the characteristic fertility pattern. There is a wide range in the types of *A. flexile* found. Some are typically small and semi-prostrate, others are taller, more upright, but still narrow. Another form is very large, and broad. When grown from spores they all look similar, which suggests their growth is as much a product of their environment and nutrition as genetically determined.

4.3: Frond size on herbarium specimens

The fronds that were measured for the morphometric analysis were also used to compare the heights of fronds from different localities. When *A. distentifolium* is protected from grazing and provided with adequate nutrients, it can grow up to at least one metre tall. In some localities like Caenlochan and Ladhar Bheinn in Knoydart it grows in dense stands with little other vegetation. In these sites the smaller *A. flexile* would not have a chance to establish. Where the two do grow together the *A. distentifolium* is relatively shorter than elsewhere (Table 4.3.1).

Table 4.3.1 Frond length of herbarium specimens of both *A. distentifolium* and *A. flexile* subdivided by locality and date of collection.

Locality	Mean	SE	Number	Range (cm)
Glen Prosen *A. flexile* recent	16.1	1.7	16	9-33
Glen Prosen *A. flexile* 1850s	28.8	4.1	10	14.5-55
Glen Prosen *A. distentifolium* recent	38.3	5.5	18	18-93
Ben Alder *A. flexile* recent	21.6	2.1	14	14-35.5
Ben Alder *A flexile* 19th Century	18.8	1.8	13	10-30
Ben Alder *A. distentifolium* recent	29.3	2.1	16	23.5-43
Creag Meagaidh *A. flexile* recent	17.1	2.6	5	17-27
Bridge of Orchy *A. flexile* recent	30.4	1.7	34	14-57
Bridge of Orchy *A. distentifolium* recent	45.8	2.6	35	28-90
Beinn Eibhinn *A. flexile* recent	14.6	1.8	7	11.5-24
Beinn Eibhinn *A. distentifolium* recent	24	2.6	11	15-40
Scottish *A. distentifolium* non-*A. flexile* sites	46.7	4.3	18	27-83
non-Scottish *A. distentifolium*	56.9	3.0	60	28-139

A sample of Scottish fronds from sites where *A. flexile* has not been found gave a mean of 46.7 cm. This mean is slightly larger than the mean for *A. distentifolium* at any of the sites where *A. flexile* has been found, the nearest being Bridge of Orchy with a mean of 45.8 cm. This is unusually large for *A. distentifolium* in *A. flexile* habitats but the *A. flexile* here is also larger than anywhere else, 30.4 cm.

The Beinn Eibhinn *A. flexile* was on average the shortest, 14.6 cm, together with the plants from Glen Prosen, 16.1 cm. A comparison with the original Glen Prosen

A. flexile specimens shows a wide discrepancy between the length of present-day specimens and the length of original fronds which was 28.8 cm. The range for the originals is much larger, 14.5-55 cm compared with 9-33 cm today. This is a significant difference ($t_{34} = 3.19$, $P = 0.003$). Only the largest plants found at the present: 33cm, are similar to the original mean size: 28.8 cm. Herbarium specimens might not be an accurate representation of a population as a collector would be more likely to collect a "good" specimen rather than a comprehensive range. Nevertheless, the Glen Prosen fronds from the 1850s are of a size that cannot be compared with any specimens that could be collected from the present population. They are comparable with fronds from Bridge of Orchy. This is the only area presently known that has such tall fronds and the present-day mean height and range are both almost the same as the 1850s Glen Prosen mean height and range (Figure 4.3.1). The Glen Prosen habitat has changed and might have suffered from acidification (3.5).

Ben Alder *A. flexile* plants from the 19th century do not greatly differ from the present dimensions: 18.8 cm compared with 21.6 cm for recent specimens. Unlike Glen Prosen the 19th century plants were slightly smaller and the difference is not significant ($t_{35} = 1.02$, $P = 0.33$).

The non-Scottish *A. distentifolium* has the largest mean, 56.9 cm, but the range of 28-139 cm included the size of fronds found in Scotland and does not exclude the possibility of coexistence with *A. flexile*-sized plants. In Norway, Odland (1995) measured fronds from 11-150 cm high so that the full range *of A. distentifolium* sizes occur elsewhere with a potential niche for *A. flexile*. Although there might be suitable habitats for *A. flexile* in other countries, it has only been found in Scotland.

4.4: Number of cells in the annulus

Some species like the three *Polypodium* found in Britain have different ranges for the number of thick-walled cells in the annulus (Page, 1997). When sporangia were examined for the multivariate analysis, it was observed that *A. flexile* frequently had higher numbers of indurated cells than *A. distentifolium*.

The earlier samples to be measured, which included Scottish *A. distentifolium* and *A. flexile*, a few of the European, Scandinavian and the North American *A. distentifolium* in the Royal Botanic Garden Edinburgh herbarium, had only ten counts on sporangia. The samples collected in the field and the herbarium specimens from Oslo and Vienna all had thirty counts. The sporangia were sampled from the nearest point to the broadest part of the frond possible. One frond each of *A. distentifolium* and *A. flexile* from Bridge of Orchy were sampled at the top, middle and base with little overall difference. The *A. flexile* frond had a mean of 15.6 cells at each point. The A. *distentifolium* sample had a mean of 15.2 cells at the top and middle and a mean of 14 cells at the base.

Overall, the *A. flexile* samples had a higher number of cells in the annulus than the *A. distentifolium*, but there was variation between populations. Some of the *A. distentifolium* plants at sites where *A. flexile* has been found had a higher mean numbers of cells, as seen in the example counted at three points on the frond. There is a significant difference between the number of cells in the annulus of Scottish compared with non-Scottish plants ($t_{123} = 4.1$, $P = <0.001$) and between Scottish *A. distentifolium* at *A. flexile* sites, and Scottish *A. distentifolium* where *A. flexile* has not been found ($t_{48} = 4.8$, $P = < 0.001$) (Table 4.4.1).

Table 4.4.1 Mean number of indurated cells in the annulus of *A. distentifolium* with the mean maximum and mean minimum of the range showing a range from 10 to 17.

	Mean ± SE	Minimum	Maximum
Central Europe n = 15	12.3 ± 0.2	10.5	14.6
Scandinavia n = 37	12.6 ± 0.1	10.5	14.9
North America n = 6	11.8 ± 0.3	10.6	13.6
Glen Prosen n = 4	13.4 ± 0.2	10.8	16.0
Ben Alder n = 17	12.7 ± 0.2	10.5	15.5
Beinn Eibhinn n = 11	12.9 ± 0.3	10.8	15.9
Creag Meagaidh n = 3	13.5 ± 0.8	11.0	17.0
Scottish Highlands n = 14	11.6 ± 0.3	10.4	13.8
Bridge of Orchy n = 33	13.2 ± 0.1	11.1	16.6

The eighty-nine *A. flexile* samples scored had a mean number of 15.8 (SE 0.2) cells while the 121 *A. distentifolium* from all sources had a mean of 12.6 (SE 0.1). This was significantly different ($t_{208} = 17.2$, $P = <0.001$).

One unexpected observation was the apparent difference between the number of cells in the annulus of *A. flexile* from Glen Prosen. There is a small difference between the number of cells in the annulus of recently collected fronds compared with those that were mostly collected in the 1850s and 1860s (Table 4.4.2). This difference is not significant ($t_{19} = 0.66$, $P = 0.51$). Ben Alder has similar numbers of cells in either sample. The comparison between recent and historic herbarium specimens suggest a further line of investigation and might well reflect a response to changes in the atmosphere and the environment. It is also not known why different locations have means that vary, as seen in the comparison between present-day Ben Alder and Glen Prosen (Table 4.4.2).

Table 4.4.2 Mean number of indurated cells in the annulus of *A. flexile* with the mean maximum and mean minimum of the range showing a range from 12 to 24 cells.

	Mean ± SE	Minimum	Maximum
Glen Prosen 1995-96 n = 14	17.8 ± 0.4	14.8	24.1
Glen Prosen 1850-55 n = 8	16.3 ± 0.4	12.9	22.4
Ben Alder 1995-96 n = 17	14.6 ± 0.5	12.4	20.3
Ben Alder 1800s n = 9	14.7 ± 0.7	12.3	16.2
Beinn Eibhinn n = 7	15.6 ± 0.5	12.8	20.3
Creag Meagaidh n = 4	16.6 ± 0.2	13.0	22.0
Bridge of Orchy n = 33	15.1 ± 0.2	12.2	19.2

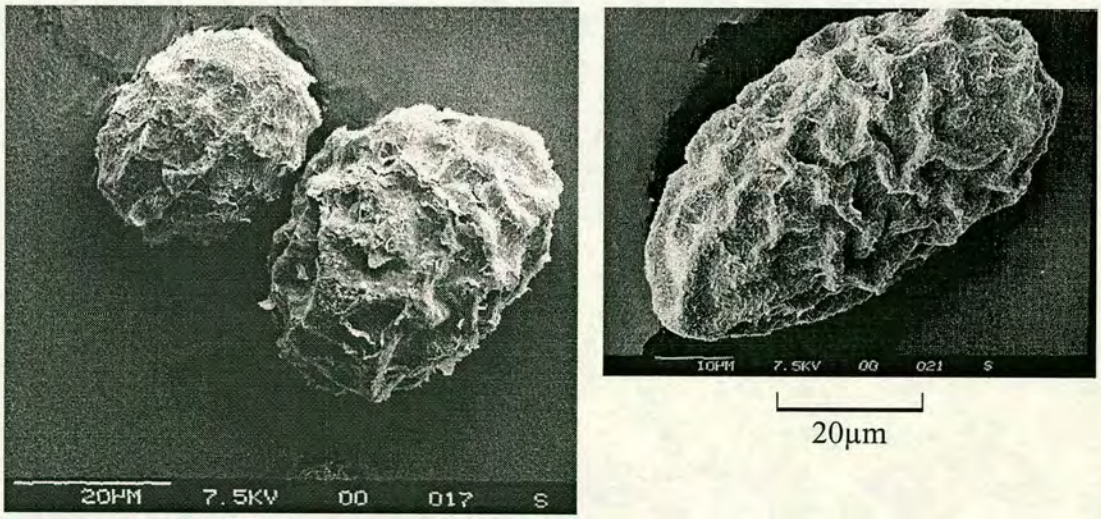
Although the mean number of indurated cells varies between location there is nevertheless a difference between the values obtained for *A. flexile* and *A. distentifolium*. The lowest number of cells for *A. distentifolium* is usually ten or eleven (Table 4.3.1), while *A. flexile* normally has a minimum of twelve or thirteen (Table 4.3.2). It is very unusual for *A. distentifolium* cells to range beyond twenty, but *A. flexile* does so frequently. It would not be adequate to confirm the identification of *A. flexile* from one count on one specimen, but the number of

indurated cells in the annulus suggest a useful character for the microscopic determination of this taxon, which has not hitherto been recognised.

4.5: Spores

A. flexile has a mean spore size of 34.1 (SE 0.4) µm with a mean maximum and mean minimum range from 25-48 µm. *A. distentifolium* has a mean spore size of 32.3 (SE 0.4) µm with a mean maximum and mean minimum range from 25-45 µm. Their size is significantly different. ($t_{76} = 3.3$, $P = < 0.001$) although the actual measurements are very close. The mode for *A. flexile* is 36 µm and for *A. distentifolium* is 32.8µm. Visual examination of the spores suggests a greater variation of volume in *A. flexile* and the *A. distentifolium* spores look more uniform with fewer very large spores to vary the mode. This size difference and its effect on growth is further developed in Sections 5.2, 5.3 and 6.1.

In appearance, the spores are very similar. They have varying development of ridges, and an angular or smoother surface of the perispore, which is unrelated to either taxon (Figure 4.5).

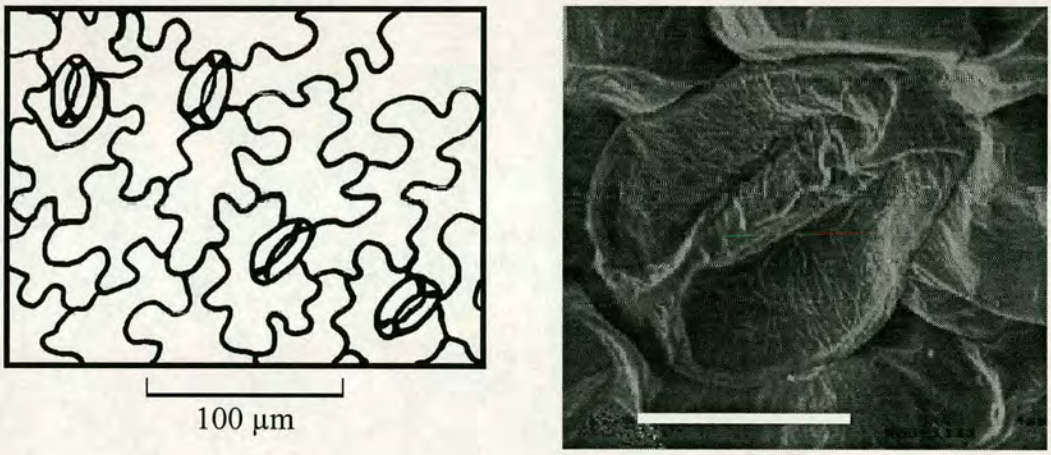


(a) (b)

Figure 4.5 Scanning electron micrographs of Ben Alder *A. distentifolium* (a) and Ben Alder *A. flexile* (b) showing variation in the definition of the ridges and in spore volume which is found in both taxa.

4.6: Stomata

Fern stomata are found only on the underside of the frond. There are few counts of stomatal frequency for ferns, a typical example that is often quoted is *Pteridium aquilinum* with 85 stomata mm^{-2} (Meidner and Mansfield, 1968) or *Osmunda regalis*: 67, and *Phyllitis scolopendrium*: 59 stomata mm^{-2} (Willmer, 1983). There was no indication as to the point on the frond at which the stomata were counted. Ferns in open situations have a larger number of stomata and this is illustrated by the *Pteridium* with a high score, and *Phyllitis*, typically growing in deep shade, has the lowest score of the three quoted. Van Cotthem (1970) classified fern stomata into five types and these *Athyrium* stomata correspond to the polocytic type where the stoma is attached to the side of a single cell that is often horse-shoe shaped (Figure 4.6a). The adjacent cells link at the end of the guard cells, or not more than half way along the guard cells



(a) (b)

Figure 4.6a Drawing based on a scanning electron micrograph of the underside of a fresh pinna of *A. distentifolium*. **4.6b** SEM of stomata of *A. distentifolium* on a herbarium specimen. The scale bar represents 20 µm.

Scanning electron micrographs of the stomata were taken (Figure 4.6b) and ten of each taxon were measured. There was little variation observed, hence the small sample. The *A. distentifolium* stomata had a mean length of 49.4 (SE 1.07) µm and a range from 42-54 µm that was very similar to *A. flexile* 48.8 (SE 1.05) µm with a range from 44-54 µm. This suggested that size does not offer an indication of differences between these taxa.

Fully expanded fern fronds were collected at the two field sites in July and kept overnight in the refrigerator. A latex medium used for making dental moulds, was mixed in small quantities and spread in pinnae-sized areas on a labelled sheet of paper. The dry fern pinnae were pressed, underside down, onto the prepared areas and left until set. The soft tissues were then peeled from the mould. *A. flexile* was particularly difficult to separate due to the small size and dissection of the pinnae. The mould was then painted with clear nail varnish. When set, this was peeled off and laid on a microscope slide. A grid graticule indicating the area of one mm^2 was used at x 100 to count the number of stomata present. An area was selected as much between large veins as possible. A mean was taken from three counts on each sample.

To compare the number of stomata from the upper part and basal part of the frond, five series of counts were made for each taxon from each site. There was only a small difference between the mean number of stomata on the upper part of the frond (Table 4.6.1). It was decided to extend the sample of pinnae from the base of the frond to include ten examples of each taxon from each site (Table 4.6.2).

Table 4.6.1 Mean number of stomata mm^{-2} of five samples from the two field sites taken from near the top of the frond. There is a difference between the two sites, but little difference between the taxa within the sites.

	Bridge of Orchy		**Glen Prosen**	
	A. distentifolium	*A. flexile*	*A. distentifolium*	*A. flexile*
Mean	70.93	64.80	56.13	55.00
SE	6.16	8.27	5.75	3.73
Mean range	59-92	44-87	42-69	44-68

The small variation between the two taxa in the number of stomata counted in the upper part of the frond (Table 4.6.1) might be due to a similar degree of exposure. A greater difference was found between the scores for each site. Glen Prosen is south-facing, but is frequently in the cloud, and the apparent difference between the Glen Prosen and Bridge of Orchy sites could be explained by Bridge of Orchy ferns

generally receiving more light. However, this was a small sample and the differences between sites were not significant.

The basal pinnae might have been expected to be more similar between populations as they are in sheltered positions. With the ten samples taken from the basal pinnae, there was a smaller difference between the sites but a more marked difference between taxa (Table 4.6.2). The Bridge of Orchy measurements showed a significant difference between *A. flexile* and *A. distentifolium*, but the Glen Prosen measurements failed to be significant ($t_{18} = 1.6$, $P = 0.11$).

Table 4.6.2 Mean number of stomata mm^{-2} of ten samples from the two field sites taken from the base of the frond. There is a smaller difference between the two sites, but a difference between the taxa within the sites with the Bridge of Orchy taxa showing a significant difference ($t_{18} = 2.8$, $P = 0.01$).

	Bridge of Orchy		**Glen Prosen**	
	A. distentifolium	*A. flexile*	*A. distentifolium*	*A. flexile*
Mean	60.50	47.77	54.68	43.53
SE	3.20	3.33	6.06	2.95
Mean range	47-80	28-68	27-86	27-56

Stomatal frequency scores on individual plants were very variable. The mean basal counts of the Glen Prosen *A. distentifolium* ranged from 27-86 and indicated how difficult it is to give an accurate generalised mean. The position of a frond within the clump could give a greater degree of shading and many ferns, but not all, grew among the rocks. *A. flexile*, as a smaller plant, was more likely to be sheltered by rocks and sometimes only the tips of the frond were exposed. The horizontal habit of *A. flexile* would also shelter the underside more than an upright frond. Although there appears to be a difference in the stomatal frequency between taxa this is the inevitable result of *A. flexile*'s growth form and plants of *A. distentifolium* growing in similar circumstances can range down to these scores.

Ludlow and Wolf (1975) found fern species that always grow in the shade have a higher chlorophyll content than ferns from sunny habitats, to compensate for the lower photosynthetic rates. There is a marked colour difference between *A. distentifolium*, which tends to be more yellow-green, and *A. flexile* that is usually a bluer-green, indicating it is more a shade fern. This might be associated with their growth habit, for *A. distentifolium* has a more exposed, upright form and *A. flexile* grows among rocks. This colour difference is maintained in cultivation where both taxa receive the same light levels.

Hew and Wong (1974) compared photosynthesising sun fern species with shade-loving species of ferns and found that sun ferns saturated at higher light intensities than the shade-loving, hence the need to have more stomata to give greater control. The shade ferns became saturated at only one sixth the light intensity at which the sun ferns became saturated, and this was a very low intensity. Growing in low cloud cover with diffused light this would be advantageous. Also, at higher altitudes the CO_2 levels are lower. At low concentrations of CO_2 the maximum possible rate of photosynthesis can be reached at low light intensities, which again reflects possible conditions.

On days of heavy cloud there is little difference between air and leaf temperatures and a high leaf temperature is only reached with high irradiance (Friend and Woodward, 1990). Very high irradiance can damage the photosynthetic system and bleaching occurs (Jones, 1992). These *Athyrium* must be able to adapt to days of continuous cloud cover and clear skies, although north-facing corries would be shaded from the sun for at least part of the day. On clear nights, dwarf vegetation loses heat more rapidly than tall vegetation, and this would affect *A. flexile* more than *A. distentifolium,* except they typically grow in areas with overnight cloud that will help to reduce the heat loss.

4.7: Chromosomes

Manton (1950) counted the chromosomes of all three British *Athyrium* taxa and determined that all had the same number of chromosomes. *A. distentifolium* chromosomes were counted from a root tip giving 2n = 80 and *A. flexile,* with difficulty, was counted from one squash in meiosis and was probably n = 40.

Fresh material in cultivation was used to confirm these counts and pinnae were collected at Bridge of Orchy. Sporangia were selected when well-formed and green, and taken from different parts of the frond. Cultivated specimens were best collected around mid-day on the first sunny day after a cloudy period when activity should be high. Wild material was fixed immediately in three parts absolute alcohol mixed in the field with one part glacial acetic acid. This was refrigerated overnight and placed in a freezer at -20 °C until used within a few months.

Acetocarmine stain was prepared by boiling 45% glacial acetic acid with 55% distilled water and acetocarmine stain added until saturation. A few sporangia were placed on a slide with one drop of acetocarmine stain and they were crushed with a needle. The coverslip was added and the preparation heated over a spirit lamp. The hot slide was squashed firmly between thick filter paper and immediately scanned at x 400. Photographs of the chromosomes were taken with high contrast black and white film using a green filter. While a satisfactory count was obtained from both taxa only clear photographs were taken of *A. flexile* (Figure 4.7).

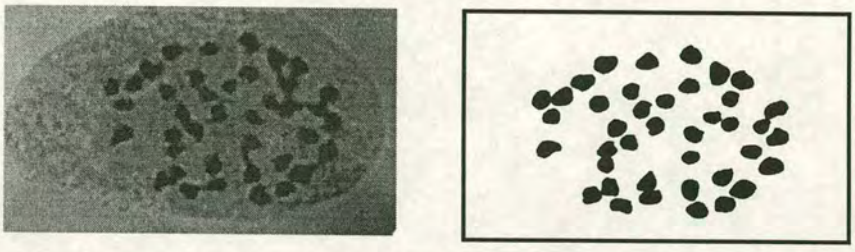


Figure 4.7 Chromosomes of *A. flexile* from Bridge of Orchy x1000

Both *A. flexile* and *A. distentifolium* appeared to have n = 40 (Figure 4.7). Pairing was regular, indicating complete compatibility. Variable levels of maturity were noted in *A. flexile* sporangia. Even on the same pinna there was range of stages seen

from fully formed spores near the rachis to the occurrence of meiosis nearer the tip of the pinna. This wide range made it less difficult with this taxon to locate sporangia undergoing meiosis at some point on the frond.

4.8: Rhizome and root structure

Both *Athyrium distentifolium* and *A. flexile* have a thick rhizome that is covered with the remains of old frond bases. *A. flexile* is generally smaller than *A. distentifolium* and the size difference between these taxa is reflected in measurements of rhizome diameter (Table 4.8.1).

Table 4.8.1 Diameter of rhizomes of *A. flexile* and *A. distentifolium* measured just below the crown, showing the generally smaller dimensions of *A. flexile*

	Mean	SE	Range	n
Ben Alder *A. flexile*	18.8	1.6	12-26	10
Creag Meagaidh *A. flexile*	19.7	1.3	15-24	21
Beinn Eibhinn *A. flexile*	16.7	2.3	12-25	5
Glen Prosen *A. flexile*	16.4	1.6	10-30	10
Bridge of Orchy *A. flexile*	24.1	1.9	16-35	10
Ben Alder *A. distentifolium*	22.5	1.8	18-35	9
Bridge of Orchy *A. distentifolium*	33.3	2.9	17-45	10
Glen Prosen *A. distentifolium*, edge	20.4	1.3	17-24	5
Glen Prosen *A. distentifolium*, centre	41.6	4.4	22-55	7

The small size of *A. distentifolium* indicated that the plants were probably not growing strongly. The Bridge of Orchy plants, where both taxa also grow together, are characteristically more vigorous. The *A. flexile* at Glen Prosen had rhizomes that were a similar diameter to those from most other sites (Table 4.8.1) but the *A. distentifolium* at Glen Prosen fell into two categories. The infertile *A. distentifolium* that grows around the edge with *A. flexile* had a mean diameter of 20.4 cm, while the larger *A. distentifolium* in the centre of the site had a mean diameter of 41.6 cm, larger than Ben Alder or Bridge of Orchy.

When plants of both taxa were observed in the field, it was very uncommon to find single crowns. On excavation, what appeared to be dispersed groups of single plants

were revealed as several crowns in a clump (Figure 4.8.1). Three complete clumps of *A. flexile* were collected, two from an eroded area at Ben Alder, and one from Beinn Eibhinn. The crowns of *A. flexile* from multiple clumps on these sites usually have four to six, sometimes seven fronds, although at Bridge of Orchy crowns had up to eight or nine fronds. When there are less than five or six fronds they usually form a single whorl. It was thought reasonable, on the rhizomes that had been collected, to age the rhizomes by counting the leaf bases on a longitudinal section of the rhizome as each representing one year's growth. Ferns with a higher number of fronds might have had two fronds almost in line for the same year, and this method would not have been possible. Up to seven frond bases remained fleshy, and up to eighteen leaf bases were counted before decay was observed. Thirteen rhizomes were cut longitudinally. On 326 mm of rhizome, 140 leaf bases were counted giving an estimated mean growth of 2.3 mm each year. Using this estimation, the branched rhizome from Ben Alder (Figure 4.8.1) represented at least thirty-three years' growth, calculating from the point where branching commenced. Even if two leaf bases represent one year's growth, this gave a minimum age of sixteen years and a range from sixteen to thirty-three. The crown would also have grown for some years before producing offsets. The original crown could have been a vegetative offset from a similar branched rhizome as occasional movement of the scree might detach parts of the rhizome.

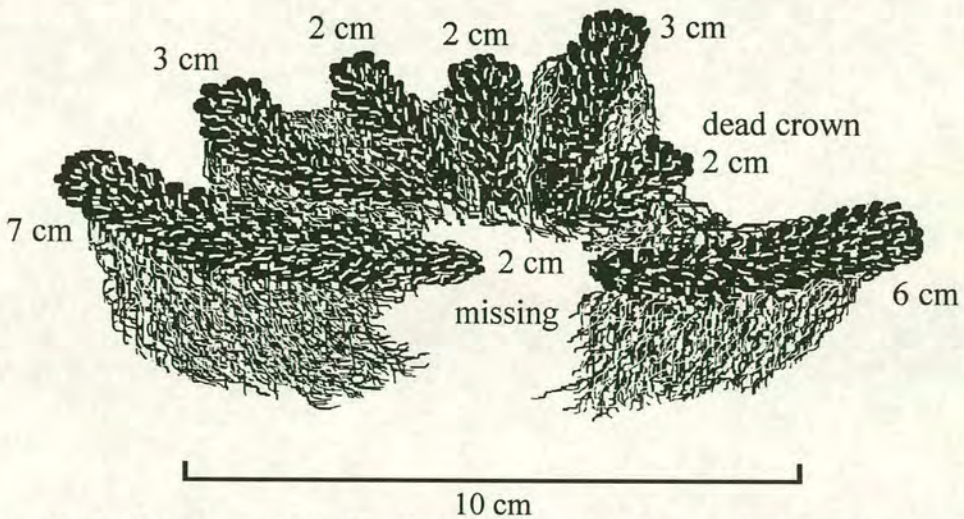


Figure 4.8.1 Rhizomes of *A. flexile* from an eroded area at Ben Alder showing six live crowns and one dead one. The rhizomes diverge away from the same point that has decayed.

Due to the more prostrate habit of the fronds, the crowns of *A. flexile* were observed to be frequently more spaced out, although this did not apply to many of the Bridge of Orchy *A. flexile* that, unusually, were more upright. *A. distentifolium* can form very large, dense, multiple-crowned clumps. At Creag Meagaidh, one particular clump of *A. flexile* took the form of a dispersed series of eleven crowns within a radius of fifty centimetres. Isozyme evidence (6.3.3) failed to detect any differences suggesting that these plants were all the same clone. As a single plant must reach some maturity before branching, and the resulting offsets would themselves branch and branch again, this clone must be of some antiquity. It would not be possible to estimate more than a minimum age for this clump, as the rhizomes were not creeping, but would have been the result of repeated offsets and branching, usually with ascending growth. As it is so uncommon to find single crowns, this suggested that most populations are composed of long-established plants and that colonisation by spores is an infrequent event.

On the Ben Alder rhizome that was cut longitudinally, one frond base that might have been five years old, had a bud with two croziers (Figure 4.8.2). This illustrated the potential for offshoots from the rhizome and would also enable new growth in the event of injury to the actual crown, through severe frosting or mechanical damage.

Figure 4.8.2
Old frond base on an *A. flexile* rhizome from Ben Alder with two croziers on the inside of the old stem and two roots growing outwards.

A root is produced at the base of each frond, and this assists in anchoring the rhizome into the mobile scree. The roots of both taxa at the Bridge of Orchy field site were observed to penetrate deep into the scree, sometimes occupying crevices between

rocks with little rooting medium present. Close to the rhizome, there were often few branches on the thong-like roots and the branch pattern at the tips was very simple (Figure 4.8.3). The finest roots were usually 0.25-0.5 mm in diameter.

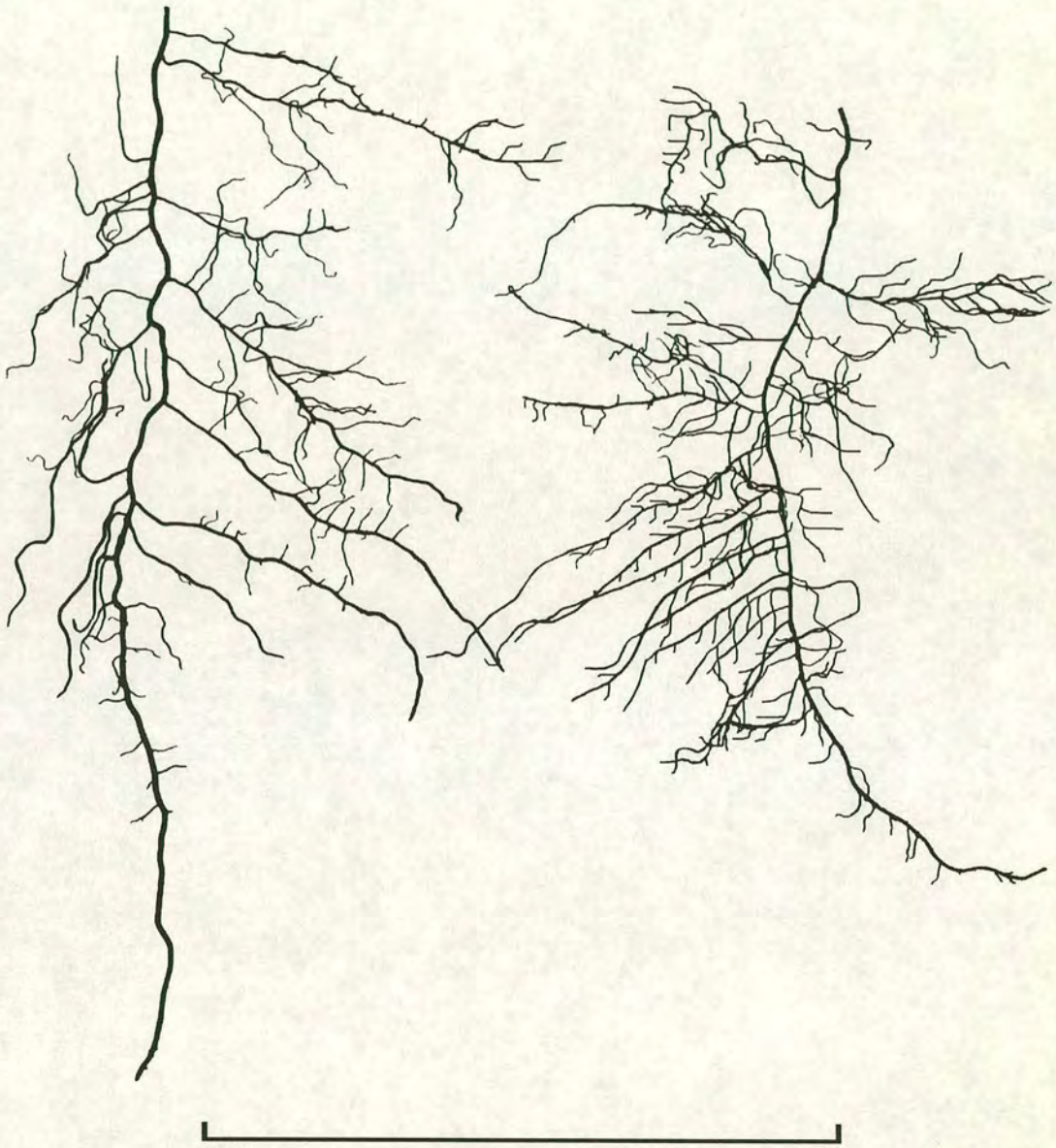


Figure 4.8.3 Roots from cultivated *A. flexile* collected in March showing a variable development of fine roots.

Both *A. flexile* and *A. distentifolium* have rooted into steep scree slopes of up to 45 ° which are often unstable. At the field study sites, several large rocks were observed to have moved during the study period. Frost action overwinter caused rock

shattering and local movement of a few centimetres was observed by rocks around monitored plants. One boulder at Bridge of Orchy, from high up in the scree, slid at least 50 m over the snow surface between April and May in 1996. The crowns of both taxa are often angled down the slope and fronds appeared from beneath creeping rocks in an environment that is constantly being modified.

Athyrium has the simplest of all fern root types which Schneider (1996) has classified as the *Lonchitis* kind of root. The rhizodermis forms the outer layer with one row of cells. A variable number of single celled root hairs arise from the rhizodermis. The cortex is composed of thin-walled cells, all much the same shape. The smaller cells of the endodermis enclose the stele (Figure 4.8.4).

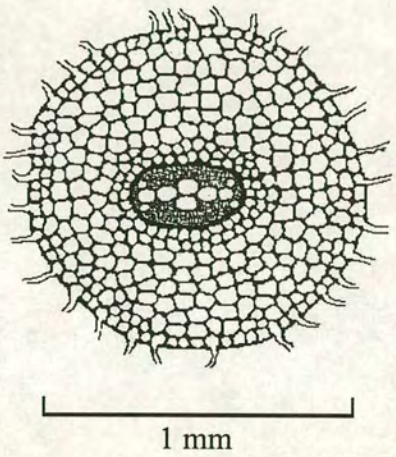


Figure 4.8.4 Cross-section of an *A. flexile* root showing the rhizodermis with the base of the root hairs, the thick cortex and the endodermis around the central stele. This was a thicker root.

4.9: Mycorrhizas

4.9.1: Introduction

Mycorrhizal associations in ferns have received little detailed attention. Boullard (1957) reported a survey of many ferns, most of them herbarium specimens, and listed the presence or absence of mycorrhizal colonisation. Of the genus *Athyrium* he mentioned only *A. filix-femina*. Within Britain, Hepden (1960) examined some native sporophytes and gametophytes but did not include *Athyrium*. In a study of pteridophytes on Rhode Island, Laferrière and Koske (1981) included two plants of *A. filix-femina*, one colonised, and one not. Berch and Kendrick (1982) examined one specimen of *A. filix-femina* in Southern Ontario and found 60% colonisation. There are three Polish papers that included *A. distentifolium* in a general study of high-altitude mycorrhizal plants. Nespiak (1953) found no mycorrhizas present, but both Dominik and Nespiak (1953) and Dominik *et al.* (1954) gave positive records of arbuscular mycorrhizas. As many populations of *A. distentifolium* and *A. flexile* in Scotland grow in a stressed, low-nutrient environment any additional source of nutrient might be especially significant. Due to the lack of information about fern mycorrhizas in general and *A. distentifolium* in particular, other sources are used to suggest factors that might be important in the development of mycorrhizas in the high-altitude environment. This discussion is followed by a report of a survey of mycorrhizal colonisation in *A. distentifolium* and *A. flexile* over two seasons at two monitored field sites, to assess the amount of colonisation and the benefits the plants might receive.

Most plants are mycorrhizal, and arbuscular mycorrhizas are the most widespread type. Ninety percent of seed plants that have been examined have a fungal symbiosis in the roots and of these an estimated 55% are arbuscular mycorrhizas (Fitter and Moyersoen, 1996). Similar estimates are not available for pteridophytes but arbuscular mycorrhizas have been recognised in the earliest land plants from the 400 million-year-old Rhynie chert (Remy *et al*, 1994) demonstrating a long association. These endophytes are unable to manufacture carbohydrate by photosynthesis or as saprophytes and receive carbon from the host plant to use in the

production of hyphae. The hyphae cover a greater area, and penetrate between smaller soil particles than the roots, making more efficient use of the carbon than the roots alone (Smith and Read, 1997). In return, the hyphae can scavenge for phosphorus (P) and other minerals like zinc and sulphur (Cooper and Tinker, 1978) and copper (Li *et al*, 1991) which are exchanged with the host plant.

Lower plants, including ferns, which lack intercellular spaces in the cortex (Boullard, 1957), have the *Paris*-type of arbuscular mycorrhizas where the hyphae usually form coils within the cell and grow from one cell to the next, producing arbuscules from the coils. Transfer of phosphorus has been thought to be through the arbuscules but the coils are probably involved as well (Smith and Smith, 1996). The coiled hyphae might offer a less efficient transfer surface for nutrients than the more abundant arbuscules of the *Arum*-type, but dense arbuscules are usually present. The *Arum*-type of mycorrhizal colonisation exploits intercellular spaces which may foster more rapid colonisation, but the *Paris*-type of arbuscules could be longer lived than the *Arum*-type as the endophyte does not necessarily need to re-colonise annually (Brundett and Kendrick, 1990). Chapin (1980) suggested that plants growing in a low-nutrient high stress environment are more suited to the slow colonisation of roots by mycorrhizal fungi that might be long-lived.

The majority of reported fern mycorrhizas are endomycorrhizas and ectomycorrhizas are apparently rare. Lohman (1927) reported ectomycorrhizas on *Onoclea sensibilis*, *Pteridium aquilinum* and *Adiantum pedatum* in woodland in Iowa. Iqbal *et al.* (1981) recorded that they found ectomycorrhizas on *Adiantum venustum* at various sites in Pakistan, where different fungi were involved within different woodlands. In the light of these findings, Berch and Kendrick (1982) particularly looked for ectomycorrhizas in southern Ontario but found none, even though the ferns they were studying grew in close association with ectomycorrhizal trees. Merryweather (pers. comm, 1997) observed a plant of *Dryopteris filix-mas* surrounded by a clearly-defined ring of basidiomycete sporocarps and suggested this might have been an ectomycorrhizal association.

Hanselwandter (1979) found that high altitude flowering plants had comparatively low levels of mycorrhizal activity, although those that were colonised grew more vigorously. Other non-mycorrhizal plants compensated by having finer roots. Körner and Renhardt (1987) found that high altitude plants developed four to five times more fine roots than lower altitude ones which gave a greater foraging area. The below ground biomass was not necessarily higher but the root architecture was modified. As an extension of this, Hanselwandter and Read (1980) observed that a fine endophyte identified as *Glomus tenue* (Greenhall) Hall was more frequent at higher altitudes where the hyphae may give finer penetration between particles. Mullen and Schmidt (1993) also found a fine endophyte but traced the increase of P in the roots and shoots of *Ranunculus adoneus* through the seasonal increase of a coarser symbiont. Due to very early growth and flowering, *R. adoneus* used P accumulated the previous season. Fresh accumulation of P was only found once the coarse endophyte had established in the new roots and had produced first arbuscules, then vesicles, ensuring a supply of P to promote new growth in the following season.

The most important nutrients have a slow rate of mineralisation at high altitudes (Hanselwandter, 1979) so that mechanisms are required to enhance their acquisition. However, Hanselwandter and Read (1981) suggested that the apparent lack of mycorrhizas at the very highest altitudes could be explained by the availability of nutrients in snow melt. Odland (1995) found a tendency for the richest stands of *A. distentifolium* to be on a steep slope of more than 25 ° which he suggested might contribute to a better supply of nutrient-rich seepage. While *A. distentifolium* would normally be free from snow by July at the latest, particularly in the east of Scotland there are frequently later lying snowbeds maintaining melt-water supplies through the summer (McVean and Ratcliffe, 1962). Some screes have only small accumulations of humus, and most of the nutrient might be derived from seeping groundwater. Boullard (1957) noted that ferns growing in rock crevices were less likely to be colonised by mycorrhizal fungi than those in soil.

Mycorrhizas also have a role to play in drought tolerance. Nelson and Safir (1982) found that drought-stressed mycorrhizal onion plants with no additional P grew significantly larger than non-mycorrhizal drought stressed onions which had been given added P. Phosphorus has a low diffusion rate in moist soil and has a lower capacity to diffuse through soil under drought conditions. Drought-stressed plants were unable to utilise the P once the roots had depleted supplies in their immediate vicinity. The hyphae in the mycorrhizal plants extended beyond the depletion zone and made better use of the limited supply of P, as the endosymbiont apparently took the P straight into the roots.

Many studies have examined the relationship between the pH of the soil and mycorrhizal activity. Read *et al.* (1976) found that in temperate grassland there were high levels of colonisation where the competition was assumed to be intense between individuals in closed communities, but was lower in fertile soil where there was less competition, the soil was deeper and there were fewer roots in contact with one another.

Cooper (1977) planted young sporophytes of *Dryopteris filix-mas* (L.) Schott. on sterilised soil with chopped fern roots and sterilised soil with autoclaved chopped roots. The plants were provided with different amounts of nutrient, especially phosphorus. Mycorrhizal colonisation was recorded for the whole root system. The P content of the fronds was analysed and root hair length, frequency and density were measured. The plants were more mycorrhizal when the soil P was low. *Pteridium aquilinum* (L.) Kuhn var. *esculentum* (Forst.f.) Kuhn and *Histiopteris incisa* (Thunb.) J. Smith showed similar responses to low soil P. The *Pteridium* had fine roots with many long root hairs and was only mycorrhizal when there were very low levels of P. *D. filix-mas* had high levels of colonisation even in the more fertile soil.

Field observations understandably present a less clear picture. James and Sheffield (1988) sampled *Pteridium aquilinum* from twelve sites, measured the dry weight of rhizome in 1 m x 1 m x 15 cm of soil and scored colonisation levels in subsamples of

roots. Soil pH, nitrogen and phosphorus were analysed. There were no correlations found between the levels of colonisation and these parameters. Similarly, Conway and Arbuthnot (1949) found that there was a limited amount of colonisation in the roots of *Pteridium* even at very different pH levels as in peaty soil at pH 4.4 and rich loam at pH 7.7. At both extremes there were still many roots not colonised at all. Hepden (1960) discovered that the earliest-formed roots of young sporophytes were more mycorrhizal than later-formed. Again, there was no correlation between soil type, moisture and level of colonisation.

Some pteridophytes, for example *Ophioglossum* and *Lycopodium,* which have a subterranean gametophyte have obligate mycorrhizal associations at that stage (Boullard, 1957). This association is less important with photosynthesising gametophytes. Cooper (1977) grew gametophytes of *P. aquilinum*, *H. incisa* and the *D. filix-mas* on soil with cultivated mycorrhizal inoculum and found they were not strongly colonised and showed little difference in development or maturity. Gametophytes grown on field soils also showed a similar response indicating that the association is not very advantageous.

Roots of both *A. distentifolium* and *A. flexile* were collected from two field sites over two seasons when they were not covered by snow. Some were lost in processing, but enough remained to enable quantification of mycorrhizal fungal populations.

4.9.2: Materials and methods

Roots were collected from blocky screes at Bridge of Orchy by removing rocks to gain access, or from the shallow soil in the monitored site at Glen Prosen. (See site descriptions of monitored field sites 3.2.1). They were collected at monthly intervals from April to October 1996, five samples each of *A. flexile* and *A. distentifolium*. Outwith this period the plants were possibly covered by snow making both access and collection difficult. At Bridge of Orchy, in May 1996, roots were dug from beneath 45 cm of snow while other plants were still two metres deep. Some samples disintegrated during processing, so further collections were made

during 1997 to span the intervals lost from the first year. As the seasons were very different these data were not compatible, but provided information from two consecutive seasons. The Glen Prosen population was so small that sampling of *A. flexile* was not maintained as regularly as from the larger site at Bridge of Orchy. Sufficient of each sample was gathered to fill a 10 x 14 cm polythene bag loosely. These were kept in a refrigerator until frozen at -20 ° within 48 hours.

The samples were kept in the freezer for up to 6 months. It was found preferable to carry out most of the processing at room temperature rather than at 90 °C as in standard methodology (Kormanik and McGraw, 1982). The cold method (Koske and Gemma, 1989) required a longer period but less attention, and there was less risk of material disintegrating. The roots were washed straight from the freezer and placed in 20% KOH in a fume cupboard. They were contained within 50 ml tubes which allowed for an appropriate volume of liquid to cover the sample. KOH cleared the roots but did not bleach them so that the roots still looked very dark when removed after 48 hours. They were washed and left overnight in 25% hydrogen peroxide 100 vols to complete the bleaching process. Following a further washing they were placed in 0.1 M HCL overnight to acidify (although 15 minutes is sufficient). Finally they were stained by a variety of methods.

Adequate staining of the mycorrhizas was achieved with 1% aniline blue in lactic acid when left overnight. Hepden (1960) used 2% aniline blue but overstaining can occur. The samples were stored in 5 mg bottles in a destaining solution of lactic acid: glycerol: water, 14:1:1. This was the simplest and safest method and gave good results. Acid fuchsin at 0.01% (Kormanik and McGraw, 1982) was used but required at least 2 hours at 90°C, and staining was variable and often poor. Brundett, Piché and Peterson (1984) used chlorazol black E 0.1% in 1:1:1 80% lactic acid: glycerol: distilled water with a destain of 1:1 glycerol : distilled water. It was found that this stain required two hours at 90°C but similar results were obtained at room temperature after 4 or 5 days. Chlorazol black E gave the best results. All the 1996

samples were stained with aniline blue and all the 1997 ones with chlorazol black E. Acid fuchsin was only used on parts of larger samples.

The finest roots were removed from each sample and mounted on slides below 22 mm x 40 mm coverslips. The roots varied considerably in diameter but no roots over 1 mm were used, and they were usually less than 0.5 mm. The roots were mounted in destaining fluid and gently squashed by tapping, to improve visibility of internal structures. Two or three slides provided enough material to record presence or absence of mycorrhizas at 100 intersections by the magnified intersection method of McGonigle *et al.* (1990). This gave a percentage for each sample of the Root Length Colonised (% RLC). Sampling points were approximately 5 mm apart. Magnification was x 150 with x 600 when required to confirm structural detail. Arbuscular mycorrhizas were determined by the appearance of vesicles or arbuscules. Non arbuscular fungi and other unidentified structures were ignored.

4.9.3: Results

As the Glen Prosen population was small, sampling was kept to a minimum. There was a marked contrast between the *A. distentifolium* plants which grew in the middle of the site, where they formed a pure stand, and the *A. flexile* plants, that were only found around the edge of the site (Figure 4.9.1).

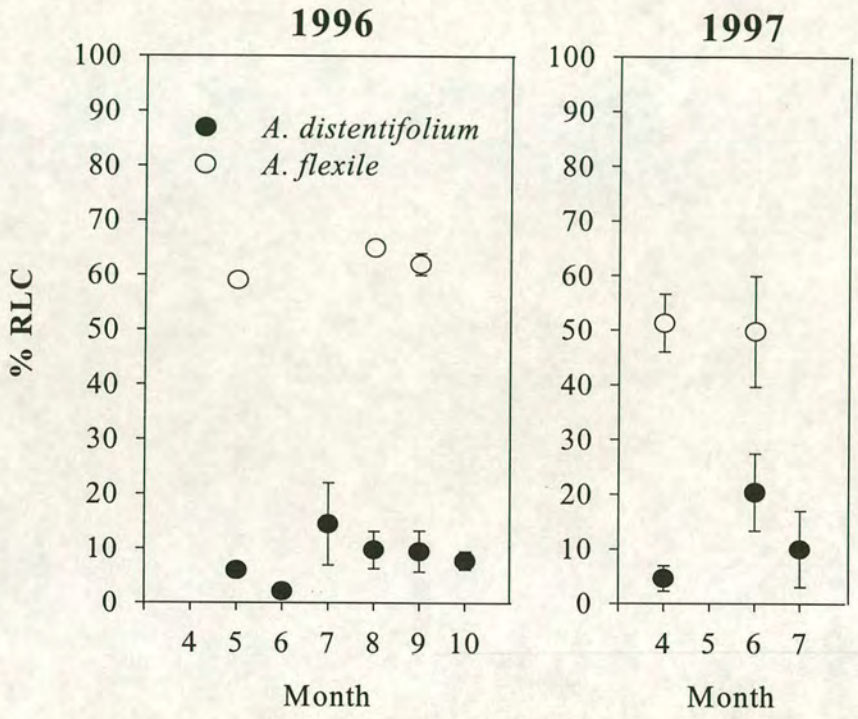


Figure 4.9.1 Glen Prosen 1996 and 1997 % RLC of *A. distentifolium* growing in the centre of the rocks and *A. flexile* at the edge, showing a higher level of colonisation in the roots of *A. flexile* (Bars are for standard error. Most samples were from 5 plants except for single plants in May and June 1996 for both taxa and August 1996 for *A. flexile*).

The *A. flexile* plants on the perimeter were growing with infertile *A. distentifolium* and a few other fern species, most of which had higher values of mycorrhizal colonisation than the larger clumps in the centre. A range of samples taken at the end of April 1997 before frond expansion had started, showed that there were already higher mean levels of colonisation in the plants growing around the edge, than in the main clump; 27.6-51.3% compared with 4.6% (Table 4.9.1).

Table 4.9.1 % RLC of the Glen Prosen ferns at the end of April 1997 showing higher colonisation scores in the roots around the edge of the colony (± standard error).

	A. distentifolium in centre	*A. distentifolium* (infertile) at edge	*A. flexile* at edge	*Blechnum spicant* *D. expansa* at edge
	12%	33%	59%	20% *B. spicant*
	0%	37%	61%	51% *D. expansa*
	3%	13%	46%	78% *D. expansa*
	0%		39%	
	8%			
Mean	**4.6%** ± 2.4	**27.6%** ± 7.4	**51.3%** ± 5.3	

Plants sampled at the beginning of June 1997 (when above-ground growth had just started) showed a mean of just over 20% RLC for the large *A. distentifolium* but nearly 50% RLC for the *A. distentifolium* and *A. flexile* growing around the edge (Table 4.9.2). The production of sporangia was noted. With this small sample the potentially fertile plants were less mycorrhizal. The only plant of *A. distentifolium* on the edge of the site which was fertile, was sampled in July and had 5% RLC. Four plants from the main clump, also sampled in July, gave values of 2% and 31% for the fertile plants, 3% and 4% for the non-fertile.

Table 4.9.2 % RLC of the Glen Prosen ferns at the beginning of June 1997, including presence or absence of sporangia on fronds. The ferns growing around the edge of the site continue to have higher rates of colonisation. * = fertile
(± standard error)

	A. distentifolium in centre	*A. distentifolium* at edge	*A. flexile* at edge
	29%	56%	74%
	17% *	40%	41%*
	8% *	63%	27%*
	43%	39%	57%
	5%*		
Mean	**20.4%** ± 7	**49.5%** ± 6	**49.75%** ± 10.1

The plants at Bridge of Orchy showed a less clear division of habitat with both

A. distentifolium and *A. flexile* growing close together showing more similar mean values and wide variation overall. The *A. flexile* roots initially had higher levels of colonisation than *A. distentifolium* but this was reversed in July 1996 (Figure 9.4.2) and the mean of both was almost identical in October 1997. There appeared to be a seasonal change with higher values during the summer.

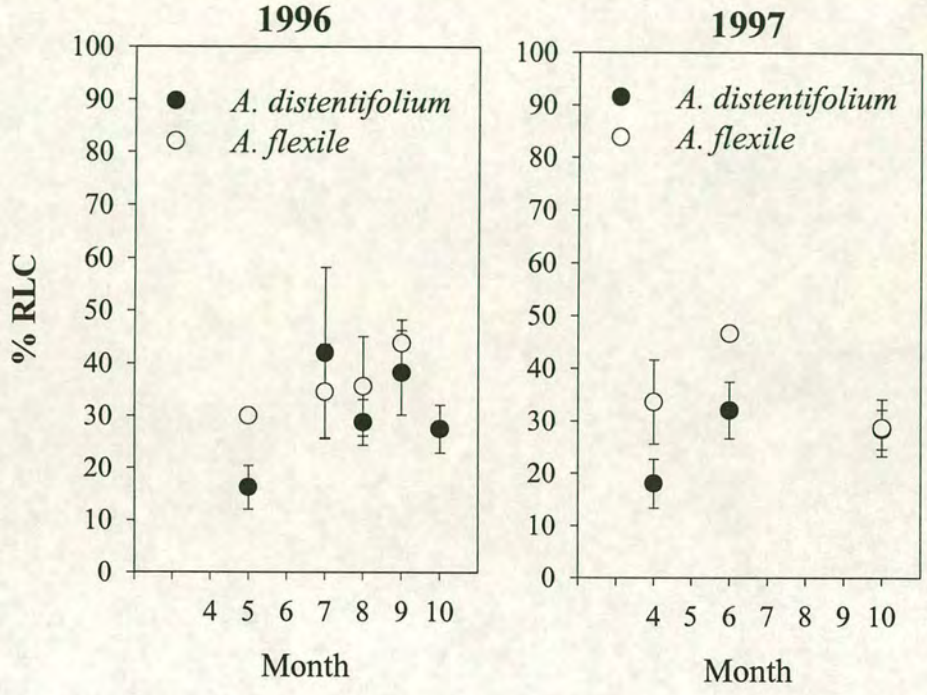


Figure 4.9.2 % RLC of *A. distentifolium* and *A. flexile* at Bridge of Orchy in 1996 and 1997 showing little difference between colonisation of the roots of the two taxa although *A. flexile* tended to have higher values. (Standard error bars. Samples were collected from five plants except for a single plant of *A. flexile* in May 1996, 10 samples of *A. distentifolium* in October 1996 and 10 samples of both taxa in October 1997).

An attempt was made to find a correlation between height, fertility and mycorrhizal colonisation in the October 1997 roots sampled from the monitored plants at Bridge of Orchy (Table 4.9.3). *A. flexile* showed very little correlation but *A. distentifolium* showed a modest negative trend that implied that the more mycorrhizal plants of

A. distentifolium were less fertile, although not statistically significant. Height showed a low positive correlation for *A. distentifolium*.

Table 4.9.3 Scores for height, mean fertility and % RLC by arbuscular mycorrhizas of marked plants of *A. flexile* and *A. distentifolium* from Bridge of Orchy, collected in October 1997. Kendall's rank correlation was used to test for a correlation of height and fertility with % RLC and this was not statistically significant. (0.5 would be necessary for τ to be significant at the 5 % level).

	A. flexile			***A. distentifolium***		
Marked Plant	Mean height cm	% Fertility	% RLC	Mean height cm	% Fertility	% RLC
1	21	100	28	32	95	9
2	22	100	10	41	95	20
3	15	100	24	55	75	41
4	21	90	37	56	95	44
5	38	100	58	55	95	19
6	24	100	43	33	0	42
7	28	100	10	35	50	23
8	21	100	42	37	50	35
9	23	90	31	55	90	32
10	26	100	4	37	95	19
τ =	-0.05	0.05		0.22	-0.42	

A sample of other ferns at Bridge of Orchy (Table 4.9.4) and Ben Alder (Table 4.9.5) showed a similar range of scores for % RLC.

Table 4.9.4 % RLC by arbuscular mycorrhizas of other ferns at Bridge of Orchy

Date collected	Species	% RLC
3.6.97	*Oreopteris limbosperma*	59
4.6.96	*Blechnum spicant*	50
2.7.96	*Gymnocarpium dryopteris*	42
	Phegopteris connectilis	52
	Dryopteris affinis borreri	10
	D. oreades	87
	D. expansa	89
	D. oreades	66
	Cystopteris fragilis	56

Table 4.9.5 % RLC by arbuscular mycorrhizas of roots collected from Ben Alder and Beinn Eibhinn in August 1996 (± standard error).

Source	% RLC	% RLC
Ben Alder 16.9.96	*A. distentifolium*	*A. flexile*
	75	66
	23	50
	43	42
	46	55
Mean	**46.75** ± 10.7	**53.25** ± 5.0
Phegopteris connectilis	82	
Beinn Eibhinn 25.9.96		
A. distentifolium	45	

The width of roots varied considerably and the thinnest roots were selected when scoring. A small sample was put into size classes to compare with the % RLC (Table 4.9.6) but no correlation was observed (Kendall's rank correlation coefficient τ = 0.01).

Table 4.9.6 % RLC by arbuscular mycorrhizas with roots collected at the end of April in Glen Prosen 1997 showing the range of diameters of the roots which were used in quantification.

Species	% RLC	diameter of root (mm)
A. distentifolium 1	12	< 0.3
A. distentifolium 2	8	< 0.3
A. distentifolium 3	0	< 0.5
A. distentifolium 4	3	< 0.3
A. distentifolium 5	0	< 0.5
A. distentifolium 6	13	< 0.5-1
A. distentifolium 7	33	< 0.3-0.5
A. distentifolium 8	37	< 0.5
A. flexile 5	59	< 0.3
A. flexile 7	61	< 0.3-1
A. flexile 8	46	< 1
A. flexile 10	39	< 0.5-1
Blechnum spicant	20	< 0.5
Dryopteris expansa 2	51	< 0.3
Dryopteris expansa 9	78	< 0.3-0.5

4.9.4: Discussion and conclusions

These results clearly show that both *A. distentifolium* and *A. flexile* can be colonised by arbuscular mycorrhizas. At Glen Prosen two comparisons can be made. The first is between the plants growing in the centre of the site and those around the edge, the second is between the performance of the two taxa, *A. distentifolium* and *A. flexile*.

The *A. distentifolium* plants, which are the sole occupants of the central site, consistently have low scores of % RLC (Figure 4.9.1). Most of the plants are vigorous, growing up to 90 cm. These large plants with deep roots might have access to sufficient nutrients and have less need of the assistance of a mycorrhizal association. Iqbal *et al.* (1981) observed that older, fertile plants were only 6.8-11.6% colonised, compared with younger sporophytes with roots colonised 10-50%. This was attributed to the development of a deeper root system. Nespiak (1953) found no mycorrhizas in *A. distentifolium* in an *Oxyrieto-saxifragetum* association and this might be an example of the absence of mycorrhizal colonisation in a high nutrient environment fed by enriched meltwaters.

An attempt was made to link the level of nutrient and the fertility of the plant with a few individual plants at Glen Prosen (Table 4.9.2). The fertile plants generally appeared to be less mycorrhizal. The distinction between the partially fertile *A. distentifolium* in the centre, and the rarely fertile marginal ones, also suggest that there may be more nutrients available to one group of plants. Fertility in some ferns is determined the previous season and depends on the availability of sufficient nutrient (Wardlaw and Sharma, 1963).

Boullard (1957) observed that ferns in wet places were less mycorrhizal. Dhillion (1993) sampled *Equisetum* from a range of sites varying from very wet to dry and found the drier sites to be most colonised with arbuscular mycorrhizas. The slope beside the ferns at Glen Prosen is flushed and it is probable that the deeper levels below the rocks were damper. This would have been a source of nutrient for the larger ferns able to benefit from it. The smaller ferns on the perimeter were in more

danger of drying out, and during the dry summer of 1995 some ferns had lost their fronds by July, although they recovered by the following year. Mycorrhizas help in stressed environments (Nelson and Safir, 1982) and may be part of the survival strategy of the plants on the perimeter.

Where *A. flexile* and *A. distentifolium* grow together around the rocks, a different response is being made to the same habitat. Here, only one *A. distentifolium* plant was fertile, while more than half of the *A. flexile* plants were. The smaller, infertile *A. distentifolium* might be maintained in a permanently juvenile state having insufficient nutrient to grow larger. The mycorrhizal colonisation was similar, implying that the *A. flexile* taxon requires less nutrient to reproduce successfully. Cultivation experiments (5.4) have demonstrated this is the case.

Both *A. distentifolium* and *A. flexile* had similar levels of colonisation at Bridge of Orchy, but the *A. flexile* plants continued to have a higher level of fertility (Table 4.9.3). Although the % RLC scores for *A. flexile and A. distentifolium* were similar, each taxon might have been deriving a different amount of benefit from this association. The trend towards negative correlation of fertility with % RLC for *A. distentifolium* at Bridge of Orchy indicated that plants that have a mycorrhizal association are less fertile, and are not growing as successfully as highly fertile plants. This is of very little significance to *A. flexile*, which is fertile in a low-nutrient environment, as already demonstrated at Glen Prosen. The Bridge of Orchy *A. distentifolium* plants may require more nutrient than the mycorrhizal association can provide. A mid-season sample might have shown a more significant distinction when colonisation was more extensive. If there is any significance in the generally higher values of % RLC of the *A. flexile* plants (Figure 4.9.2), also indicated in the small sample from Ben Alder (Table 4.9.5), it might be that *A. distentifolium* occupies more enriched pockets within the mosaic of the scree.

A variation was observed in the diameter of roots being scored in this study. With low % RLC scores only the narrower, youngest roots were colonised. Roots with

higher scores were heavily colonised in both the thinner, thicker and sometimes even the thickest roots. This implied that at least two-year-old roots could be occupied by mycorrhizal fungi. Although these fern roots were less than 1 mm, often less than 0.5 mm, they were still comparatively thick compared with many flowering plant roots. Peat and Fitter (1993) scored the colonisation of roots of flowering plants from 0.1 mm to 0.3 mm and found that roots with a smaller diameter were less mycorrhizal, and the ones that were greater than 0.3 mm were nearly all colonised. By this classification all the fern roots were relatively thick so that no relationship might be expected between the root diameter and the level of colonisation. The lack of correlation confirmed this.

The 1996 season was very late, the 1997 season was earlier and the Bridge of Orchy 1996 May-July roots might be more comparable to April-June 1997. The apparent fall in % RLC of *A. distentifolium* in Glen Prosen July-August 1996, June-July 1997 and Bridge of Orchy July-August 1996 (Figures 4.9.1 & 4.9.2) could relate to a decline in rapid frond expansion. Plants which grow very rapidly are more likely to have excess photosynthate available for mycorrhizas when 4%-20% of the net photosynthate is used (Smith and Smith, 1996). Plants would also require a higher nutrient input at this time which the mycorrhizal association could supplement. *A. flexile* showed less variation through the season, and at Bridge of Orchy in 1996 (Figure 4.9.2), the mycorrhizal colonisation continued to rise until September. This might be explained by the continuous production of new fronds which is more marked in *A. flexile* than *A. distentifolium*.

While the ferns below snowbeds will be protected from temperatures falling far below zero, temperatures will also remain low for most of the winter. If the ground is covered with snow, the surface temperature would be almost zero (3.2.3). Kimball *et al.* (1973) recorded temperatures of -0.6 °C 12 cm below the soil surface under snow cover in Utah, which was similar to 0.3 °C at 5 cm below 40 cm of snow in Norway (Odland, 1995). Without snow cover, the temperature will fluctuate more. The overwinter maximum and minimum thermometer under a rock at Bridge of Orchy

recorded temperatures which ranged from 15 °C to -4 °C October to April while there was no above ground growth. The ground might be colder without a snow cover and considerable fluctuations will occur. *Hyacinthoides non-scripta* (L.) Chouard ex Rothm. roots grow actively throughout the winter, but not at such low temperatures. Merryweather and Fitter (1998) recorded mean temperatures of around 6 °C at a depth of 14 cm during the coldest months of November to February in North Yorkshire. The alpine plant *Ranunculus adoneus* (Mullen and Schmidt, 1993) appeared to rely on the mycorrhizal colonisation of new roots before the appreciable input of P later in the very short season. As these *Athyrium* are not high alpine species they do not have such a short seasonal constraint. Mycorrhizal colonisation is presumably present all winter, but might not be very active. The presence at Bridge of Orchy of higher values of % RLC for *A. flexile* in April compared with October (Figure 4.9.2) indicated root activity well before the fronds expanded. Roots taken from beneath the snow in May 1996 had visible arbuscules so that some root growth may occur during warmer periods before the fronds have started to grow.

The wide variation in the levels of mycorrhizal colonisation indicate that these ferns are not wholly dependent on mycorrhizas for their nutrition. While deductions can be made about individual plants growing in varying edaphic conditions, the higher values of % RLC indicate that some plants might be assumed to be receiving some benefit from the association. However, mycorrhizas vary in the efficiency of P transfer and the amount of carbon used, and it is possible for carbon to be extracted without any beneficial return to the host (Smith and Smith, 1996).

There are many areas which could be investigated further. Different widths of hyphae and sizes of vesicles were noted but not quantified. Individual mycorrhizas could be trapped and identified, and then plotted through the season to compare colonisation patterns in the field. Similar structures were observed in both *A. flexile* and *A. distentifolium* and it was assumed that both taxa are colonised by the same mycorrhizas, but this is not known. Experimental work could help to explain the contribution each mycorrhizal taxon makes to the host plant, and other plants in the

community. More precise knowledge of the individual mycorrhizas involved would be necessary before drawing any further conclusions.

4.10: Conclusion

With taxonomic comparisons alone, it is possible to maintain the distinction between *A. distentifolium* and *A. flexile*. The morphometric analysis showed that there is only a small overlap between taxa. The Scottish *A. distentifolium* forms a link between the *A. distentifolium* elsewhere and *A. flexile*. This implies, that at least morphologically, some of the Scottish *A. distentifolium* has features that distinguish Scottish populations from those elsewhere in the world. While herbarium specimens might be the result of biased sampling, there is the suggestion of a size difference between Scottish *A. distentifolium* and *A. distentifolium* from other sources. Scottish *A. distentifolium* is generally smaller than elsewhere, and the populations that grow with *A. flexile* are usually even smaller.

The higher number of cells in the annulus of *A. flexile* is a newly recognised taxonomic feature. Just as some of the Scottish *A. distentifolium* appeared to be morphologically intermediate between other sources and *A. flexile*, so too, do *A. distentifolium* plants from populations with *A. flexile* present, have a higher number of cells in the annulus. The values are still nearer to the *A. distentifolium* mean than the *A. flexile* mean, but the Scottish *A. distentifolium* from sites where *A. flexile* has not been recorded, has a smaller mean, as do the non-Scottish *A. distentifolium*. None of the Scandinavian or European *A. distentifolium* had high numbers of cells in the annulus approaching *A. flexile*.

Several sets of measurements were very similar. There appears to be little significance in the size of the spores. The size of stomata is similar between both taxa. The chromosome number is the same for both *A. distentifolium* and *A. flexile*, but as *A. filix-femina* is also the same this information serves to illustrate that cross-fertilisation is possible but not does not necessarily occur, although hybrids have been recorded in Europe between *A. distentifolium* and *A. filix-femina*. (2.9.5).

The stomatal density shows variation between sites and different points on the frond. This is an example of apparent differences that can be explained by local conditions at the sites. The differences in the basal stomatal density between taxa are partly explained by a height difference, which is always maintained. This ensures that *A. distentifolium* or *A. flexile* have varying degrees of exposure because of their stature, and the growth habit seems to be genetically determined, as, with a few exceptions, *A. flexile* is smaller. The different growth forms are reflected in the lower density of stomata for *A. flexile* and the darker colour of chlorophyll suitable for a sheltered environment.

The rhizome measurements continue to show the size difference between *A. distentifolium* and *A. flexile*. *A. distentifolium* can be extremely large, but the crowns of plants that grow in close proximity to *A. flexile* are relatively smaller. In the section on mycorrhizas it was found that both taxa growing together have only small differences in mycorrhizal colonisation, but these plants made different growth responses to apparently similar conditions. At Glen Prosen, small *A. distentifolium* grew with small *A. flexile*, but usually only the *A. flexile* was fertile.

When the distribution of *A. distentifolium* and *A. flexile* were compared in Chapter 3, it was found that many of the sites where *A. flexile* was found with *A. distentifolium*, were of low-nutrient status, and not typical of many of the *A. distentifolium* habitats in Scandinavia. The morphometric analysis, which used ratios to minimise the size comparison, found that much of the Scottish *A. distentifolium* occupied a distinct position relative to other *A. distentifolium*. While there are still many similarities, and Scottish *A. distentifolium* only represents one end of a range, it continues the implication that some Scottish *A. distentifolium* is distinctive, both in habitat and also in some morphological details. It is this exceptional A *distentifolium* with a modified morphology which shares the *A. flexile* habitat.

CHAPTER FIVE From spore to sporophyte

5.1: Introduction

Previous chapters have reported observations made in the most part on plants in the field, or wild material collected for analysis, together with a description of the habitats in which populations of *A. distentifolium* and *A. flexile* are found. Many of the plants appear to be large clumps, and small, young plants were not found very frequently. To produce a sporophyte, many conditions have to be fulfilled, from the germination of the spore, through gametophyte growth and fertilisation to the mature, fertile fern. This chapter links the responses of *A. flexile* and *A. distentifolium* in an experimental setting to a range of environmental variables. Their phenology was monitored in the field and provided comparisons to set along side the laboratory work.

While *A. flexile* and *A. distentifolium* had appeared to be morphologically separate on a taxonomic examination, once experimental work had begun, the division became less clear. It became apparent that gametophytes of either taxon could make different responses to temperature and nutrients that continued to emphasise their differences. The discovery that spores from some plants of *A. distentifolium* could ultimately produce both *A. distentifolium* and *A. flexile* sporophytes made interpretation of the growth of the gametophytes more complicated. Spores from *A. flexile* plants gave *A. flexile* progeny, but spores from *A. distentifolium* could either produce only *A. distentifolium* or produce both *A. distentifolium* and *A. flexile*. While it was quite clear with sporophytes which taxon was under cultivation, it was impossible to know which gametophytes were likely to give rise to which taxon. The possible origins and implications of this are discussed at the end of the chapter.

5.2: Spore germination

5.2.1: Introduction

The number of spores produced by *A. flexile* and *A. distentifolium* is very variable. Fronds might have abundant sori, or have only a few sporangia in sparse sori. In the sites where they grow together, *A. flexile* spored more frequently than *A. distentifolium*. Both can, however, produce abundant spores. As gametophytes were so infrequently observed in the field (5.3) these spores apparently had little opportunity to grow. The arctic-alpine environment in which they must germinate raises questions about the production of viable spores in a short growing season and spores were collected from a variety of sites to compared their germination potential. It is not known whether spores would be more likely to germinate in the late summer immediately after they have been shed, or if they would meet with greater success by delaying germination until the following spring. The possible influence of daylength and the temperature requirements for germination were systematically explored under laboratory conditions.

Athyrium is homosporous, and the spores are approximately the same size, unlike some fern genera like *Ceratopteris* that have two sizes of spores with a predisposition to grow into either a male or female gametophyte (Sayers and Hamilton, 1995). Nevertheless, a variation in spore size was noted in both *A. distentifolium* and *A. flexile* (4.5) and this has possible implications for the gender of the gametophytes, a theme that is further discussed in Chapter 6. Extreme sizes of spores were sown to find out if their germination rates might vary. The influence of the substrate in germination success was tested by sowing onto gel media with different pH levels. The gradual decline in the viability of spores was noted, as some of the same spores were used in different experiments over a twelve month period. Soil samples were collected in the field to test for the presence of a sporebank and to assess the capacity of these taxa to regenerate from at least a short-term spore bank if opportunity should arise.

5.2.2 Materials and methods

a) Collection and storage of spores. Spores were collected in the field into paper spore packets that were spread out at room temperature to dry rapidly. After a minimum of a week, the spores were tapped to one end of the packet, most of the sporangia were discarded, and the spores were transferred to small plastic tubes with a screw top. They were labelled with the species name, location and date of collection. These were stored in the cold room at approximately 4 °C until sown.

b) Sowing batches of spores. The end of each tube of spores was covered with four layers of lens tissue, firmly taped around the top. The spores were sown through the layers of tissue by tapping the end of the tube as it was held over the gel dish at a distance of about 20 mm. The number of taps was adjusted according to the abundance of spores in the tube, to give an even sowing density which was checked through a microscope (x 40). Thereafter, the tubes were returned to the cold room in a small, individually labelled, sealed plastic bag, until used again. The batches of spores used in repeated germination experiments had all been germinated after collection and only those with reasonably high germination rates were used as listed in Appendix 2.

c) Sowing individual spores. Spores for individual sowing were selected with a sterilised needle under a stereo microscope at x 80. The needle was sterilised in alcohol and then washed with distilled water. Large and small spores were defined as being greater than 40 microns, or less than 30 microns. They were either placed singly in dishes of 5 x 5 cells, or in the combination of a pair of large spores, pair of small spores, or a large with a small spore. The spores used were all collected at Bridge of Orchy in 1995, two sources of 100 each of *A. flexile,* from marked plants *A. flexile* 3 & 6, and 100 *A. distentifolium*, from *A. distentifolium* 5.

d) The standard germination medium A standard gel medium with a pH of 3.8 was selected for the germination of most spores. The formula for this and the limited amounts of pH 5.8 and 7.0 that were used for the substrate experiments was taken

from Dyer (1979, Appendix 3). Stock solutions were mixed and kept in the cold room at approximately 4 °C until used. Phytagel, which is similar to agar, was mixed into the made-up solutions at 7 g l^{-1} of solution that was autoclaved for 40 minutes. Once the solution had cooled below 60 °C an anti-fungicide, was added (Nystatin, 10 000 units ml^{-1}). Once cooled, the made-up 5-cm Petri dishes, or plates with 5 x 5 individual cells, were stored in sealed, new, plastic bags in the cold room.

e) Germination responses. To assess the germination response to daylength, spores from two *A. flexile* and three *A. distentifolium* plants were sown with a 24, 18, 12 and 6 hour photoperiod at 15 °C and scored after fourteen days. This was repeated once. Dark germination was tested by sowing spores onto the gel medium and the dishes were wrapped in foil and placed in the incubators at 15 °C. Two samples were examined weekly and then discarded. Spores were twice sown on gels at 15 °C with three different levels of pH: 3.8, 5.8 and 7.0. They were scored at 14 days.

f) Scoring germination. Three counts of 100 spores were made in each dish to be scored. The germination percentage was the mean of these three values. The appearance of the initial rhizoid was taken as successful germination, not merely swelling of the spore. From 25 °C to 15 °C, observations usually extended over fourteen days, scoring on alternate days from the fourth day after sowing. No germination was observed at three days. At 10 °C, germination was slower and further counts were made at weekly intervals. Each experiment had one dish for each different set of spores.

g) Incubators and the temperature gradient bar. Most of the spores were germinated on the top shelf in one of three incubators with a temperature of 15 °C and light levels of approximately 135 µmol m^{-2} s^{-2}. Several series of spores were also germinated in a temperature gradient bar. The design for this was based on equipment used in Sheffield (Furness and Grime, 1982). The ten-millimetre-thick aluminium bar had a reservoir beneath each end, in contact with the base. Water from a heated water bath circulated beneath one end, and cooled water was circulated

beneath the other. A gradient from 5 °C to 35 °C was obtained. The bar was in an insulated aluminium box with glass covers, contained within a growth cabinet with 24 hour tungsten and fluorescent light. Light levels were 850 μmol m^{-2} s^{-2}. The Petri dishes were in direct contact with the base plate and bubble-wrap was used to adjust the gradient in specific areas to allow more samples at a similar temperature on the gradient. Temperatures in the temperature gradient bar fluctuated by up to two degrees above and below the required temperature as the room temperature varied with the weather and other equipment in use. The temperature was monitored by battery-powered probes embedded in Petri dishes filled with plain gel. These sat on the base plate and recorded maximum and minimum temperatures, which were logged every time a series of scores was recorded with the various treatments.

h) Cultured soil. Samples cultured for determining the presence of a sporebank, were collected in June from soil as near the surface as could be found in the scree and placed in a new polythene bag in the field. The soil was spread over 5 cm^2 pots of sterilised compost in a layer 5 mm thick, watered with distilled water, covered with cling-film and placed in the incubator.

5.2.3 Results

a) Viability of spores

Samples of spores (Tables 5.2.1 & 5.2.2) showed that most plants bearing fertile fronds can produce at least some viable spores, ranging from only 5 to nearly 100%. The 1995 Ben Alder spores collected at the beginning of August were not fully mature, which gave lower germination. The 1996 Ben Alder spores were collected one week later in a different season and were better developed. The spores from Bridge of Orchy usually germinated well, and unlike the other sites most of the plants were fertile.

Table 5.2.1 Percentage of spores which germinated from 51 plants of *A. distentifolium* collected from 4 different localities in 1995 and 1996.

Source	Date collected	Number of plants	Mean % germination	SE	Range
Ben Alder	8.8.95	7	**38**	12.7	10-97
Bridge of Orchy	16.8.95	10	**85**	6.5	43-97
Glen Prosen	14.8.96	5	**97**	2.8	90-99
Ben Alder	16.8.96	11	**73**	7.0	26-97
Beinn Eibhinn	25.8.96	9	**76**	7.2	31-98
Bridge of Orchy	18.8.96	9	**91**	3.8	66-99
Mean			**76.7**	8.6	

Table 5.2.2 Percentage of spores which germinated from 62 plants of *A. flexile* collected from 4 different localities in 1995 and 1996

Source	Date collected	Number of plants	Mean % germination	SE	Range
Glen Prosen	14.9.95	5	**94**	2.6	85-98
Ben Alder	8.8.95	8	**51**	10.7	5-96
Bridge of Orchy	16.8.95	10	**94**	3.2	65-99
Glen Prosen	14.8.96	8	**83**	7.1	50-100
Ben Alder	16.8.96	12	**67**	8.3	23-99
Beinn Eibhinn	25.8.96	9	**80**	6.3	38-99
Bridge of Orchy	18.8.96	10	**94**	2.8	70-99
Mean			**80.4**	6.2	

b) Daylength

The spores exposed to various daylengths showed a difference in the rate of germination for spores from different sources. This accounted for more variation than the difference between various photoperiods, although both were statistically significantly different. (Figure 5.2.1). The difference in photoperiod was significant at the 5% level.

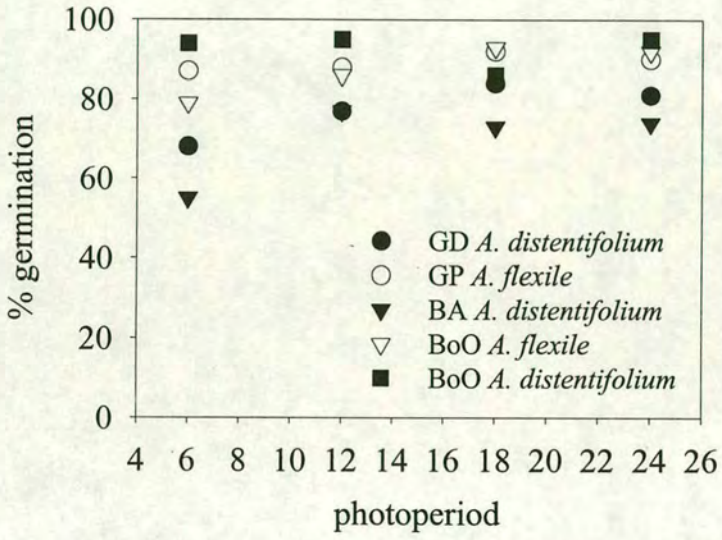


Figure 5.2.1 Germination of five sets of spores at four different daylengths, 6, 12, 18 and 24 hours. Only at six hours was a marked difference becoming apparent, particularly for two sets of spores. Key: GD = Glen Doll, GP = Glen Prosen, BA = Ben Alder, BoO = Bridge of Orchy.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Individual ferns	1419.7	4	354.92	12.78	0.0002	3.26
Photoperiod	307.4	3	102.46	3.69	0.04	3.49
Error	333.1	12	27.75			
Total	2060.2	19				

Dark germination was tested three times. In the first experiment, the same batches of spores were unwrapped, briefly examined and then wrapped up in the foil until the next examination two days later. These showed some germination after three weeks, apparently responding to a very brief exposure to light. When a series of samples

were examined at two-weekly intervals, and then discarded, no germination occurred after three months. This was repeated with the same results.

c) Temperature of germination

Each germination experiment on the temperature gradient bar was at a slightly different range of temperatures and valid comparisons can only be made between the germination of spores that were all in the same experiment. Spores from different localities responded to varying temperatures with slightly different timing. At 25 and 20 °C, spores from the Glen Prosen area and Bridge of Orchy (Figures 5.2.2 a-d) had nearly reached their maximum germination within six days. The Beinn Eibhinn spores (Figures 5.2.2 e-f), usually took longer, eight days to the equivalent stage. This is seen more clearly at the lower temperatures where Beinn Eibhinn spores required 21 days at 15 °C to reach their maximum germination, while even the 10 °C spores in the other four sets had reached this in 14 days. At 10 °C the Beinn Eibhinn spores had not reached their maximum germination within 21 days.

A set of spores was maintained at 5 °C for 16 weeks with no germination. The temperature was then raised to 6-7 °C owing to the operation of other equipment, and the spores germinated. Growth was extremely slow and after a further 8 weeks the experiment was concluded.

At the opposite extreme, spores were maintained at 30 °C. Some of these germinated but soon died. A more detailed trial used seven sets of spores at four higher temperatures. Beyond 31°C there was no germination. After seven days the spores were reduced to a lower temperature, but still did not respond. After 2 weeks they were presumed dead. At the three lower temperatures, 30-31 °C, 27-28 °C and 24-25 °C, three sets of spores did not germinate at 30-31 °C (Table 5.2.3). Of the others that did germinate, they did not have such high germination scores as those at the slightly lower temperatures. Even after several weeks, there was little increase in germination. The spores at 27-28° C had the fastest initial germination but the 24-25 °C spores eventually caught up or exceeded this level of germination. As before,

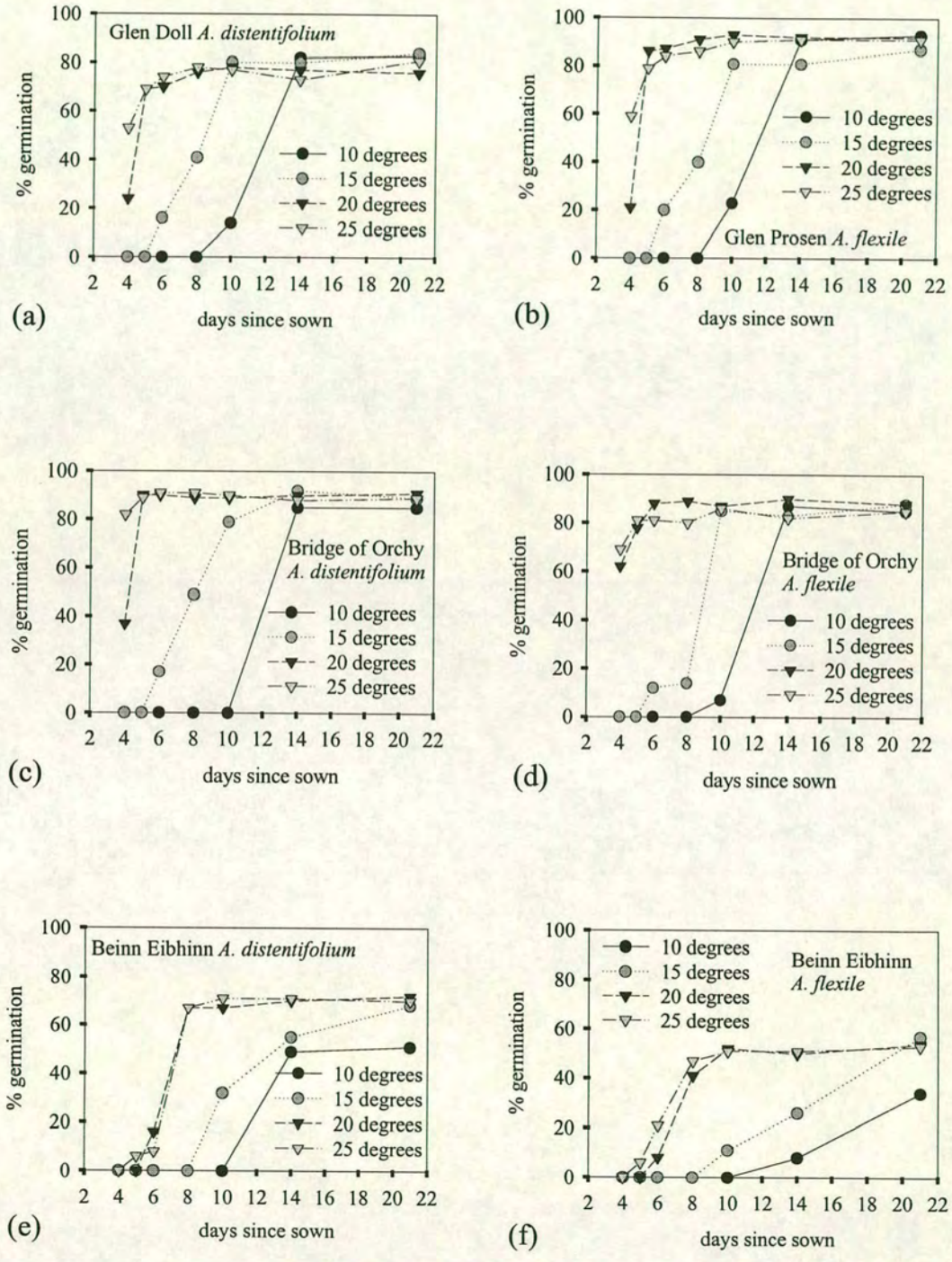


Figure 5.2.2 Germination of six sets of spores showing approximately similar responses from *A. distentifolium* and *A. flexile*. The spores from Beinn Eibhinn (e & f) showed delayed germination, particularly at the higher temperatures.

different sources provided slightly different responses but it appears that germination occurred most readily at around 25 °C.

Table 5.2.3 Germination for seven sets of spores at three high temperatures scored at 7 days with additional later scores at the lowest temperature. The apparent reduction in germination in the later scores for two populations is due to sampling error.

Range of temperatures	G. Prosen *A. flexile*	B. Alder *A. distent*	B. Alder *A. flexile*	B Eibhinn *A. distent*	B. Orchy *A. distent*	B. Orchy *A. flexile*	G. Doll *A. distent*
30 - 31° C	70 %	31 %	0 %	0 %	82 %	77 %	0 %
27 - 28° C	95 %	75 %	30 %	57 %	98 %	96 %	77 %
24 - 25° C	98 %	73 %	13 %	67 %	91 %	80 %	75 %
24 - 25° C + 12 days	96%	75 %	23 %	70%	90 %	85%	81%

d) Volume of spores

Variation noted in the volume of spores and individual spores were placed in cells singly or in pairs, in various combinations to monitor their growth. The germination of the different sizes of spores was noted (Table 5.2.4) but there was no significant difference in the germination potential of any particular size of spore.

Table 5.2.4 Germination percentages at three weeks of 150 large (>40 μm) and 150 small (< 30 μm) spores after 21 days at 15 °C showing no significant difference ($t_5 = 0.46$, $P = 0.66$) with a paired t-test on an arcsine transformation of percentages.

	Large spores	**Small spores**
A. flexile No. 3	96 %	80 %
A. flexile No. 3	64 %	92 %
A. flexile No. 6	68 %	88 %
A. flexile No. 6	96 %	92 %
A. distentifolium	100%	96 %
A. distentifolium	92 %	100 %
Total germinated	**88%**	**84%**

e) Substrate

Spores were sown on gels with three different levels of pH (Table 5.2.5). There was no indication that pH had a significant effect on germination.

Table 5.2.5 Mean % of spores germinated after 14 days at 15 °C on gel at 3 different pH levels showing little difference between pH levels.

	pH 3.8	pH 5.8	pH 7.0
Glen Prosen *A. flexile*	91 %	88 %	87 %
Bridge of Orchy *A. flexile*	92 %	88 %	90 %
Glen Doll *A. distentifolium*	86 %	84 %	73 %
Ben Alder *A. distentifolium*	73 %	69 %	71 %
Ben Eibhinn *A. distentifolium*	42 %	56 %	54 %
Bridge of Orchy *A. distentifolium*	84 %	92 %	94 %

f) Spore longevity

Some of the same spores were used for experiments over twelve months from the time they were originally collected. During this period, there was an overall decline in their germination potential (Figure 5.2.3). A two-way analysis of variance showed a significant difference in the viability. After an initial rapid decrease in viability the germination rates did not appear to be declining as rapidly.

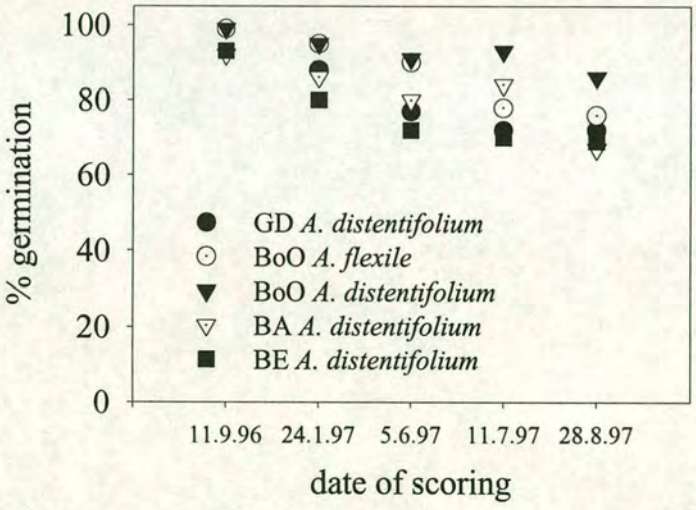


Figure 5.2.3 Germination at 14 days and 15 °C of a set of spores scored over a 12 month period showing a significant decline in germination potential. Key: GD = Glen Doll, BoO = Bridge of Orchy, BA = Ben Alder, BE = Beinn Eibhinn

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
months since collected	1509.44	4	377.36	20.04	< 0.001	3.01
individual ferns	763.84	4	190.96	10.18	< 0.001	3.01
Error	302.36	16	18.84			
Total	2574.64	24				

g) Sporebanks

Soil samples were collected from Bridge of Orchy, Ben Alder and Glen Prosen before the current season's spores had been released. Nearly all produced gametophytes from which sporophytes of both *A. flexile* and *A. distentifolium* grew, in addition to other pteridophytes and mosses. Two Glen Prosen samples produced only bryophytes.

5.2.4 Discussion and conclusions

All the spore collections produced at least some spores that were able to germinate. The poor germination percentages of some batches can be explained as fronds were gathered before the spores were fully mature.

The precise daylength did not seem to be very significant in stimulating germination, as this occurred even with a short photoperiod. There was a greater variation between the responses of spores from different populations. A range of germination percentages would always occur in separate experiments and all but the six hour daylength scores came within the range that might be expected. The slightly reduced germination at six hours possibly implied that spores might germinate more readily in the longer daylength of early summer, rather than during a mild period mid-winter. A sample of all the spores was sown soon after collection. The high germination percentages obtained implied that it was not necessary for the spores to experience a cold period.

The pH of the substrate appeared not to affect germination as such. As with the daylength, the germination percentages for each batch of spores varied slightly and there was no indication that the germination at each pH level was anything other than natural variation. A larger sample of both taxa might have shown consistent differences. Greater extremes of pH could have influenced germination. The lowest pH values in the wild might be derived from excessively acidic melt-waters (3.5) but low temperatures linked with this run-off would possibly not encourage germination.

Large and small spores seemed to have an equal chance of germinating although this was not tested at different temperatures. It is possible that with a greater volume large spores may cope better with extreme high temperatures and be less vulnerable to desiccation, at least during a short period.

For alpine taxa it is perhaps surprising to find such rapid germination at comparatively high temperatures. Hill (1971) found that species of ferns which inhabited open areas could germinate over a higher range of temperatures, 10-35 °C, than the 10-30 °C of woodland ferns. *Thelypteris palustris* Schott, for example, in an open marsh had an optimum growing temperature range of 25-30 °C compared with 15-25° C for the woodland species *Adiantum pedatum* D. Don. The open-habitat species also had a higher tolerance of light intensity. Although the *A. distentifolium* habitat in Scotland is an open scree, many populations in other countries are at the upper limit of woodland, (3.3.1), and the boulders themselves would provide some shade. Germination up to, but not exceeding, temperatures of 30 °C suggested that *A. flexile* and *A. distentifolium* might be more adapted to woodland conditions.

The temperatures recorded by the data-logger at Glen Prosen showed that for parts of the day, temperatures could rise to 30 °C, but on only two occasions reached towards the lethal 35 °C (Figures 3.8a & b). While germination can occur at 6 or 7 °C there is the potential to exploit higher temperatures if available. Temperature was the most significant factor in germination, but at the highest temperatures the percentage of germination was reduced before a lethal level was reached. There was very little difference in the rate of germination at 20 and 25 °C and even at 15 °C germination was only a few days delayed for most of the spores. The mean temperatures recorded by the data-logger for the growing season was around 10 °C (Table 3.1) and there was no difficulty in initiating germination at this temperature, but the rapid response to higher temperatures suggested that germination might more readily occur during periods of exceptional warmth.

Dyer and Lindsay (1996) compared the germination of a range of species from a variety of habitats. With *Woodsia ilvensis* (L.) R. Br. *W. alpina*, (Bolton) S. F. Gray. *Asplenium adiantum-nigrum*, *Athyrium filix-femina* (L.) Roth, *Dryopteris cristata*, (L.) A. Gray, *Gymnocarpium robertianum* (Hoffm.) Newman, *Osmunda regalis* L, *Phyllitis scolopendrium* (L.) Newman, *Pteridium aquilinum* (L.) Kuhn, and *Thelypteris palustris*, they found an optimum growing temperature of 20-25 °C, with only slightly slower germination at 15 °C and eventual full germination at 10 °C. None of these species had germinated at 5 °C after 57 days. These results are very similar to the germination rates of *A. distentifolium* and *A. flexile*. The only species that was very different was *Cystopteris dickieana* Sim. This taxon germinated faster than the others previously listed and at 10 °C was nearly as fast as at the higher temperatures. More interestingly, germination occurred at 5 °C. As *Cystopteris fragilis* (L.) Bernh. will produce new fronds in a mild period mid-winter, *Cystopteris* might have the ability to make use of any warmer period outside the usual growing season. *A. distentifolium* and *A. flexile*, did not germinate during sixteen weeks at 5 °C and it appears that they require a period of warmth to initiate growth.

Minimum germination temperatures are not often quoted for ferns, but *Pteridium aquilinum* was reported to have partially germinated in the dark in five weeks at 1-2 °C (Conway, 1949). Only beneath extended snow-cover might such temperatures be maintained, and then the temperature would probably be nearer zero. Red light is necessary to initiate germination, and blue light that might penetrate snow cannot do so (Raghaven 1989). *A. distentifolium* and *A. flexile* did not germinate in the dark.

The density of sowing might have had an effect on germination, as Smith and Robinson (1971) found that very high densities of *Polypodium vulgare* L. had lower germination. However they were using very dense cultures and no difference was observed in these experiments with *A. flexile* and *A. distentifolium* between germination in the denser areas or on the more thinly distributed edge.

During the course of twelve months the spores diminished in viability, some batches slightly faster than others. The cultured soil samples indicated that spores can survive in the soil for nearly twelve months, probably much longer. They might have a better chance of survival in the soil than stored dry in the cold room.

Viable spores have been found in the soil at depths of up to 125 cm (Dyer and Lindsay, 1992), but there is no means of establishing the age of these spores. The failure of dark germination in *A. distentifolium* and *A. flexile* indicated they may be able to maintain a spore bank for some time, unlike *Pteridium aquilinum* which can germinate in the dark (Conway, 1949).

Some of the spores were badly affected by mould that eventually smothered the culture. Bell (1958) found that some spore cultures germinated much more readily than others and discovered a fungus was present that made germination faster. Presumably this had a similar nutritional effect to mycorrhiza or a chemical was emitted which had a beneficial effect. This was not found in any of the cultures here.

5.3: Growth of the gametophyte

5.3.1: Introduction

During the three years of this study, no gametophytes were found in the field. The habitat generally had little exposed soil. In any small areas of erosion or bare ground, seen at Ben Alder and Glen Prosen, the first coloniser was the thallose liverwort, *Pellia epiphylla*. This is frequently found in gaps between the rocks and a local vegetative source is available to cover the ground rapidly. Only once, in July 1986, was a dense patch of large gametophytes of *A. distentifolium* seen in the field; at Caenlochan in Angus, growing beside a stream. The snow lay late that year and there was still a large snowbed a few metres upslope. The gametophytes had produced the first sporophytes and must have grown over at least the previous season and over-wintered beneath the snow. Sato (1982) observed that summer green ferns had prothalli that overwinter as gametophytes and may not be fertilised in the first full season. He found that sporophytes were produced towards the end of the second season and overwintered as very small plants. This was possibly the pattern followed by *A. distentifolium*.

In view of the lack of field evidence, gametophytes were cultured to compare their growth at different temperatures, their response to variations in the specific nutrients provided and growth rates at different pH levels. Gametophytes derived from the germination experiments of large and small spores were grown on to compare their mortality and growth rate.

5.3.2: Materials and methods

a) Growth medium

The gametophytes in the first and second temperature experiment were a continuation of the spores germinated at the same temperatures on pH 3.8 (5.2). For the nutrient experiment the medium was mixed as for pH 3.8 with one each of the four basic solutions (Appendix 3) omitted. This gave the normal medium minus magnesium, calcium, iron or phosphorus with potassium. One batch was also made

up without the Nystatin anti-fungicide. There were also the three pHs, 3.6, 5.8 and 7.0 (Dyer, 1979 Appendix 3),with pH 3.8 forming the control. This gave eight different treatments.

b) Sources of gametophytes grown at different temperatures and with different levels of nutrient

Spores that had been germinated on the temperature gradient bar were grown on for twelve weeks. Two growth experiments are described. The first used four pairs of replicates, the second used seven pairs. In the nutrient experiment, there were eight treatments for each of *A. flexile* and *A. distentifolium*, and there were eight replicates of each taxon. The eight replicates were pairs of *A. flexile* and *A. distentifolium* from Beinn Eibhinn, Ben Alder, Glen Prosen, Glen Doll, Creag Meagaidh, Fuar Tholl and Bridge of Orchy. If possible, pairs were from the same locality. In some cases if a sufficient supply of spores was not available, pairs were used from different localities. Some were fresh spores from the 1997 season, others were the same as those which had been used for 12 months. (Appendix 2 gives the sources of the spores and the experiments in which they were used).

c) Gametophytes derived from different sized spores

Spores were sown singly, or as pairs in separate cells (5.2) approximately 5 mm apart, and were grown as gametophytes for ten weeks. Of the spores that were sown on their own, 18 large and 18 small spores survived for the ten weeks. Of the pairs of spores in various permutations, 20 pairs survived for ten weeks. Their growth was measured with a stereo microscope, magnification x 40, by measuring across the widest point from lobe to lobe of the prothallus. The results from *A. flexile* and *A. distentifolium* were amalgamated as they showed the same general trend.

d) Incubators

The spores for the nutrient experiment were germinated and grown in three incubators at 15 °C in which they were rotated between the top and middle shelf and the different incubators at weekly intervals. Each pair of eight treatments was kept

together in the same incubator, but randomly arranged. The light levels were low, 135 μmol m^{-2} s^{-1}, and linear growth was usually observed. This was the same for all the samples but their growth was less than it could have been under higher light conditions. Forty gametophytes in each dish were measured at the broadest point, lobe to lobe. They were selected by following a line from the edge of the dish and thalli were measured if they were flat enough to see entire.

e) The temperature gradient bar

The gametophytes grown at different temperatures were maintained on the temperature gradient bar at 10, 15, 20 and 25 °C. They were allocated a position so that each set was in a different arrangement across the bar. As the larger samples involved three rows of Petri dishes across the gradient bar, these were rotated at fortnightly intervals. The light level was 850 μmol m^{-2} s^{-1} which stimulated good growth. For the last five weeks of the second experiment the tungsten component was disconnected due to an electrical problem, leaving only the fluorescent tubes with 625 μmol m^{-2} s^{-1}. Forty heart-shaped gametophytes were measured from the outside edges of the two lobes, for each dish.

f) Problems with the gel

It was found that the gel growth medium stored in made-up Petri dishes in the cold room appeared to change over time. This gave different results in successive experiments for gametophytes from the same source at similar temperatures. Dyer (1979) recorded that a medium of pH 4.1 rose during use to pH 5.5. This was not tested, but might help to explain the inconsistent results. The size of the gametophyte might have been a response to sowing density and efforts were made to provide visually equivalent densities. Despite this, some spores were sown more thickly than others. Nevertheless, examples were noted of small, well dispersed gametophytes, at the same temperature as larger densely distributed thalli that had made full use of the space available. As all spores for a range of treatments were sown at the same time each dish would be comparable with the other spores from the same source grown at different temperatures.

g) Problems with the spores

Cultivation experiments (5.4) showed that some *A. distentifolium* plants produced both *A. flexile* and *A. distentifolium* progeny. Where possible, germination and gametophyte growth experiments used spores from *A. distentifolium* that had been grown to the sporophyte stage and was known to produce only *A. distentifolium* sporophytes. There were not enough spores from known sources for the nutrient and second gametophyte size experiment, which had many replicates. Three batches of fresh *A. distentifolium* spores from the field were used, but sporophytes produced from this *A. distentifolium* in culture have since demonstrated that one of these sources was able to produce both *A. flexile* and *A. distentifolium*, and this will have influenced the results.

5.3.3: Results

a) Temperature response

There was considerable variation between the vigour of gametophytes grown from spores from different plants. A plant of *A. flexile* from Ben Alder produced large prothalli considerably larger than any others (Table 5.3.1) with a mean of 4.6 mm at 15 °C. The temperatures that had been suitable for germination were too high for sustained growth, and some of the gametophytes at 25 °C died. Most of the gametophytes grew faster at 20 °C, except the Glen Doll *A. distentifolium* and Ben Alder *A. flexile* that responded better to a lower temperature. A considerably longer period at 10 °C would have given extended data but even within twelve weeks the Glen Prosen *A. flexile* made relatively fast growth and reached a mean size of 1.3 mm when other gametophytes were between 0.3 and 0.5 mm.

Table 5.3.1 First temperature experiment. Width of gametophytes grown from the spores of four *A. flexile* plants and four *A. distentifolium* plants after 12 weeks, showing variations in size at different temperatures. BE = Beinn Eibhinn, BO = Bridge of Orchy, GP = Glen Prosen, GD = Glen Doll, BA = Ben Alder.

Source	20 °C	15 °C	10 °C
BE *A. flexile*	1.9 mm	1.6 mm	0.5 mm
BE *A. distentifolium*	1.7 mm	1.1 mm	0.3 mm
BO *A. flexile*	2.1 mm	0.8 mm	0.4 mm
BO *A. distentifolium*	1.6 mm	1.0 mm	0.3 mm
GP *A. flexile*	1.8 mm	1.7 mm	1.3 mm
GD *A. distentifolium*	0.9 mm	2.2 mm	0.5 mm
BA *A. flexile*	2.5 mm	4.6 mm	0.3 mm
BA *A. distentifolium*	1.4 mm	0.8 mm	0.3 mm

The mean width of these same *A. flexile* and *A. distentifolium* gametophytes (Figure 5.3.1) suggested differences between their growth rates at all temperatures with *A. flexile* generally growing faster.

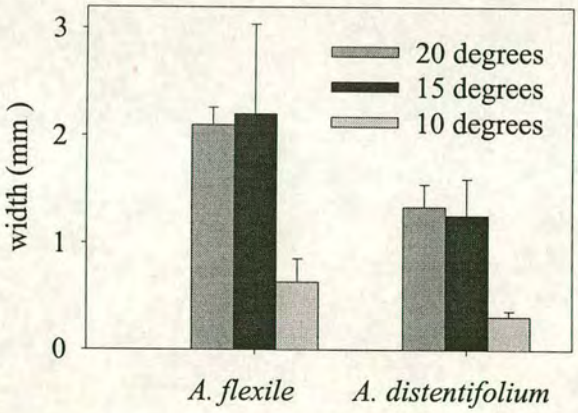


Figure 5.3.1 First temperature experiment. The mean width of four sets of *A. flexile* and four sets of *A. distentifolium* gametophytes grown at 20, 15 and 10 °C for 12 weeks showing the larger mean width of *A. flexile* gametophytes at all temperatures (standard error bars).

The second and larger temperature experiment with seven *A. flexile* sets of gametophytes and seven *A. distentifolium* produced ambiguous results (Figures 5.3.2 a-d) which were partly related to the Ben Alder *A. distentifolium* spores that produced mixed progeny. None of the gametophytes grew well at 25 °C (Figure 5.3.2 a) but the *A. flexile* gametophytes were significantly larger. There was only a slight

difference at 20 °C (Figure 5.3.2 b) but at 15 °C (Figure 5.3 c) there was little difference and the *A. distentifolium* gametophytes were eventually larger than the *A. flexile*. The slower growth at 10 °C (Figure 5.3.2 d) was only just beginning to produce a separation between the two taxa.

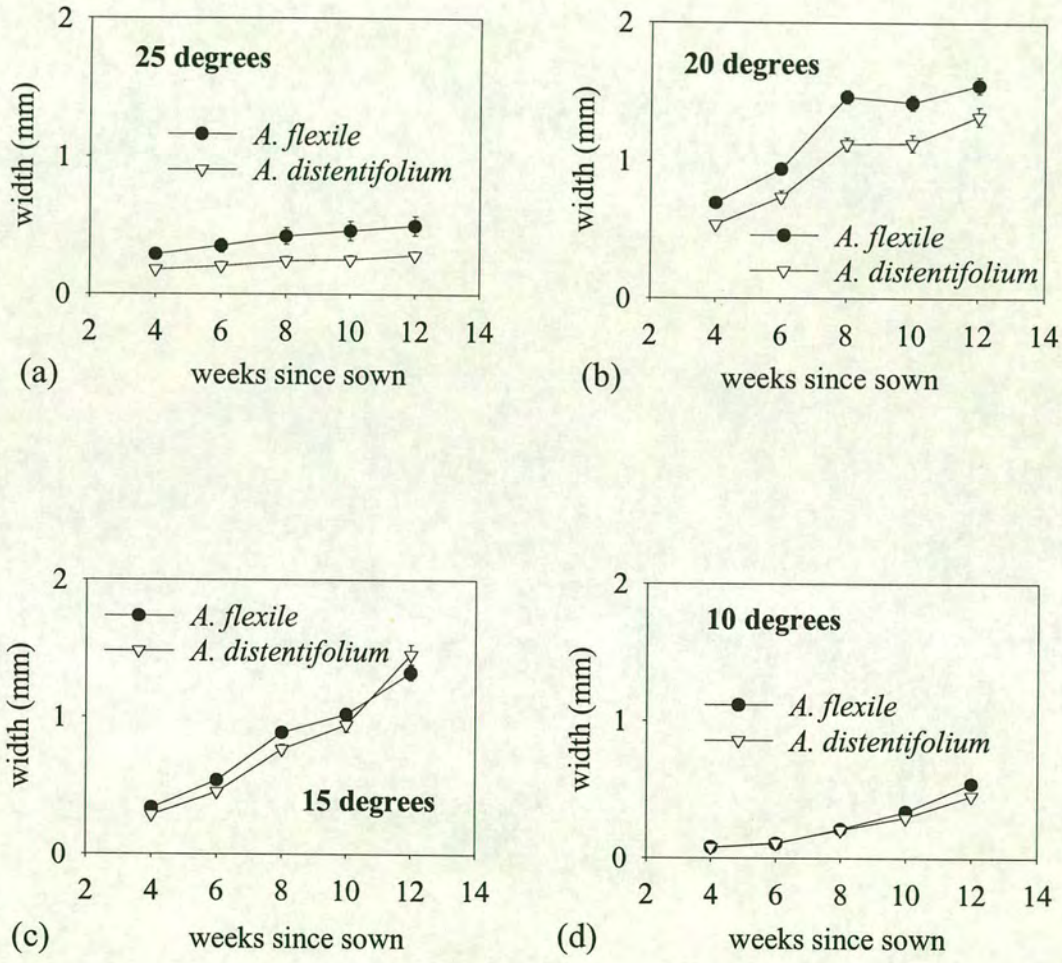


Figure 5.3.2 a-d Second temperature experiment. Mean values of 6 sets each at 25 °C (a) and 7 sets each of *A. flexile* and *A. distentifolium* (b-d) at 20, 15 and 10 °C measured for 12 weeks. Only (a) is significantly different: $t_{10} = 3.75$ $P = 0.012$ (standard error bars).

b) Response to nutrients

Eight replicates of each taxon were sown on 8 different nutrient treatments (Figure 5.3.3). Overall, *A. flexile* grew larger, with much variation between individuals. The *A. distentifolium* gametophytes grew larger than *A. flexile* without the anti-fungicide, but not significantly (Table 5.3.2). *A. flexile* showed a decline in vigour with increasing pH while *A. distentifolium* was not influenced. Magnesium is essential for healthy growth and all gametophytes were similarly affected by its absence. Iron made little difference but the mean for *A. distentifolium* was almost half that of *A. flexile* when calcium was absent, a highly significant difference (Table 5.3.2). The absence of phosphorus and potassium made little difference to *A. distentifolium* but *A. flexile* grew better without these nutrients than under any other conditions, including the balanced nutrients provided by the standard pH 3.8 medium.

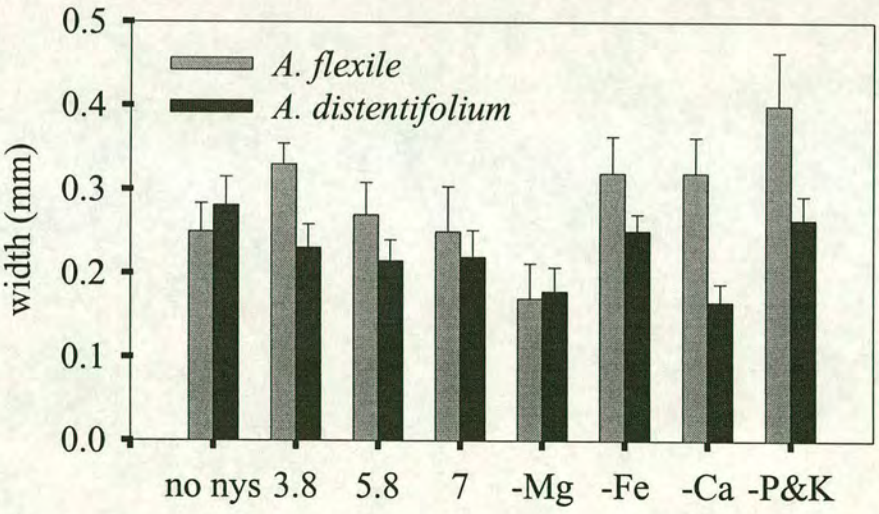


Figure 5.3.3 Mean values of 8 replicates of *A. flexile* and *A. distentifolium* gametophytes grown on a range of gel mediums: without the Nystatin anti-fungicide, at three different pH levels and the standard pH 3.8 with one each of the nutrient solutions omitted (standard error bars).

Table 5.3.2 The results of t-tests between the mean sizes for the 8 sets of *A. distentifolium* and *A. flexile* with 14 degrees of freedom showing significant differences at the 5% level for pH 3.8 and <1% level without calcium.

	No Nystatin	pH 3.8	pH 5.8	pH 7	-Mg	-Fe	-Ca	-P & -K
t =	-0.85	2.37	1.23	0.28	0.1	1.52	3.26	1.94
P =	0.41	0.03	0.23	0.77	0.91	0.15	0.005	0.07

c) Gametophyte success relative to spore size

Survival of the gametophytes grown from large and small spores was monitored over an eight week period. After four weeks, 76% of the larger spores had a living gametophyte, and 63 % of the small spores (Table 5.3.3). After eight weeks, 50% of the gametophytes derived from larger spores had survived and 36% of the smaller. They did not suffer from desiccation, and although algae were present, some gametophytes grew regardless. With only one or two spores in the cell, although they might interact, there would not have been enough competition to stop the gametophyte growing. An arcsine conversion of these percentages just failed to show a significant difference in a paired t-test between the surviving gametophytes at four weeks ($t_5 = 2.263$, $P = 0.07$) but did show a significant difference after eight weeks at the 5% level as more gametophytes grown from larger spores had survived (Table 5.3.3).

Table 5.3.3 Percent survival of gametophytes grown from large and small spores from an original sample of 150 large and 150 small spores showing that more of the gametophytes grown from large spores survived. Significant at eight weeks in a paired t-test $t_5 = 3.049$ $P = 0.03$ (on an arcsine conversion).

	4 weeks		**8 weeks**	
	Large	Small	Large	Small
A. flexile No. 3	92%	68%	76%	68%
A. flexile No. 3	58%	36%	46%	16%
A. flexile No. 6	68%	72%	36%	24%
A. flexile No. 6	88%	72%	56%	36%
A. distentifolium	92%	76%	56%	42%
A. distentifolium	80%	84%	36%	40%
Total survived	**76 %**	**63 %**	**50%**	**36%**

Eighteen large and eighteen small spores grown in separate cells initially demonstrated a size difference for gametophytes as predetermined by the size of spore (Figure 5.3.4). But after ten weeks the small spore source gametophytes had grown as large or larger than the gametophytes from large spores and there was no longer any difference in their size. When two spores were placed together they interacted with one another. With two large or two small spores in the same cell, the first to germinate generally became the larger gametophyte. Spore size was a factor in determining the size of the gametophyte in the early stages of growth (Figure 5.3.4), but without competition, the original spore size eventually became irrelevant. When two spores were placed together, the order of germination was of greater significance. The difference between the size of gametophytes grown from large and small spores paired together was highly significant at ten weeks, having increased through time (Figure 5.3.5).

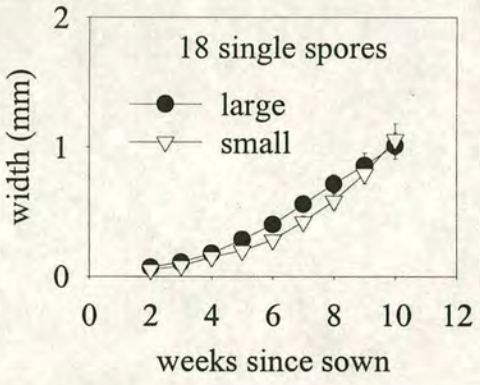


Figure 5.3.4 Width of gametophytes grown from 18 large and 18 small spores of *A. flexile* and *A. distentifolium* combined showing a significant difference ($t_{34} = 2.7$ $P = 0.01$) by five weeks after which the size difference diminishes.

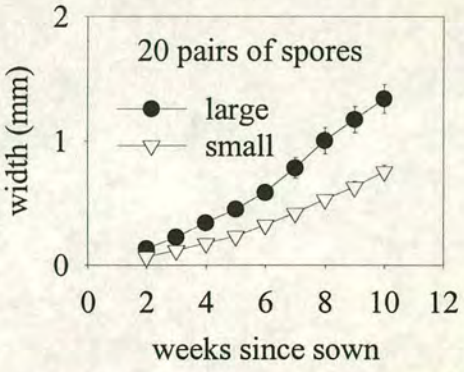


Figure 5.3.5 Width of gametophytes grown from 20 pairs of spores showing the larger gametophytes remained significantly larger and the smaller continued to grow, but less vigorously than if they had been alone (paired t-test $t_{19} = 3.28$ $P = 0.004$).

Spore size affected the probability of producing a larger gametophyte thereafter, as nine out of twelve large spores produced larger gametophytes when paired with a smaller spore (Table 5.3.4).

Table 5.3.4 Pairs of large and small spores which were sown together showing the relative size of gametophytes produced after 10 weeks *D* = *A. distentifolium*, *F* = *A. flexile*

	Large spore source		Small spore source		Same size pairs	
	D	*F*	*D*	*F*	large *F*	small *F*
Number of spores	7	5	7	5	12	4
Large gametophyte	5	4	2	1	6	2
Small gametophyte	2	1	5	4	6	2

5.3.4: Discussion and Conclusions

Bare ground that provides habitats for gametophytes can occur by various means. Red deer have been observed on a fragile slope at Ben Alder, eroding the scree, and exposing the soil. Freezing and thawing in the winter can dislodge unstable rocks which plough into a scree and open up the surface. The same process can also push large boulders out from the slope leaving a sheltered niche. When this area of bare soil does become available, colonisation may only occur if several conditions are fulfilled.

Watson and Vazquez (1981) made comparisons between the establishment of gametophytes of *Woodsia scopulina* D. C. Eaton and *Athyrium filix-femina*, by examining the composition of the substrate, the pH, sunlight, exposure and nearby plant associates. It was found that *A. filix-femina* required a more shaded habitat for the gametophyte than the sporophyte. Gametophytes initially established on areas of bare ground where there were no bryophytes, as they could not compete. Once sporophytes were established, they were able to grow above both bryophytes and flowering plants, for mature sporophytes were found surrounded by bryophytes and some litter. Young sporophytes of *Woodsia scopulina* were observed to have killed other gametophytes nearby. This suggested the presence of a toxic compound leaching from the fern fronds, or possibly the roots (Munther and Fairbrother, 1980).

As *A. distentifolium* habitats have abundant supplies of long-lasting litter, this might explain the absence of gametophytes on well-colonised screes. Munther and Fairbrother (1980) also found evidence of inhibitions that operated on gametophytes of their own and other species with differences between populations and fronds of different ages. *Dryopteris intermedia* and *Osmunda cinnamonea* interacted at the gametophyte stage and a more mature gametophyte made the others perpetually juvenile during its lifetime. It cannot be assumed that *A. distentifolium* is the only taxon attempting to establish and other species might also be involved with potentially complicated interactions. The difference between spores grown in isolation and spores grown as pairs illustrated the effect that the first-germinated gametophyte had upon the other. As the size difference was maintained for several weeks after germination, this would allow the initially larger gametophyte to reach maturity first, and possibly affect the other gametophyte.

Sayers and Hamilton (1995) found that homosporous *Ceratopteris richardii* spores normally produced male or female gametophytes but in single-spore cultures they nearly always became hermaphrodite. With same-size spores, both were hermaphrodite. When the pairs were different sizes the largest spore was sometimes female, the smaller male, but this only occurred in 11 out of 23 spores where this size difference existed. Nine out of 12 large spores produced large gametophytes with *A. flexile* and *A. distentifolium*. The size difference may not be very important, but could have more influence than has been realised.

Gametophytes have a chance of success where there is sufficient distance from nearby mature sporophytes, no toxic compounds inhibiting germination and subsequent growth, lack of competition from bryophytes or other gametophytes, adequate daylength, nutrients and temperature. Possibly gametophytes from spring-germinated spores have a better likelihood of survival if it is necessary to overwinter and grow on in a second season. The daylength germination experiment (5.2) suggested this might be the case. The low light levels in the incubators produced unsatisfactory growth from many of the gametophytes, particularly those in the

nutrient experiment where even the gametophytes with full nutrients produced linear gametophytes. Filamentous growth is produced with only red light, low light levels or long wavelengths and blue light is necessary for normal planar growth (Raghaven, 1989). The poor growth of these gametophytes suggested that they normally require considerably higher light levels. The change in light provision in the temperature gradient bar might also have affected growth as there was a distinct change in the growth measurements of the second experiment from eight weeks (Figure 5.3.2 a-d).

A. distentifolium and *A. flexile* gametophytes made varying responses to different temperatures and this indicated another factor which might influence the success of individual gametophytes in a mixed population derived from several parent plants. Although field temperatures would fluctuate widely, these growth responses (Table 5.3.1) indicated different optimum temperatures. If *A. flexile* gametophytes can grow faster and larger there is a greater probability that they will produce successful sporophytes instead of *A. distentifolium*. Field measurements from Glen Prosen suggested the mean summer growing temperature could be around 10 °C (3.1.3). The second temperature experiment confirmed that growth at this temperature was slow, for example, 0.6 mm for *A. flexile* and 0.35 mm for *A. distentifolium* in twelve weeks, but one batch of spores from Creag Meagaidh had archegonia within twelve weeks and most of the gametophytes from other sources had antheridia (6.1), so that sporophytes could have been produced soon after twelve weeks, even at these lower temperatures. If *A. flexile* can become fertile at lower temperatures this taxon would have a competitive advantage.

The nutrient experiment showed that some individuals of *A. flexile* responded especially well to a low-nutrient medium and this implied an advantage over *A. distentifolium* if attempting to establish in low-nutrient localities. *A. distentifolium* maintained a similar size on the three different pH gels, but *A. flexile* showed a slight decline in size as the pH increased. Although germination was not affected by pH, subsequent growth apparently was, and *A. flexile* gametophytes appear to be less likely to grow large gametophytes on richer soil. Neither taxon grew well without

magnesium, but *A. distentifolium* was strongly affected by the absence of calcium, while this did not appear to be important for *A. flexile*. This suggested that the gametophytes might establish more successfully in a nutrient regime that will also be suitable for the sporophyte.

A. distentifolium had better gametophyte growth without the Nystatin anti-fungicide, while *A. flexile* grew less well without it. This could be because gametophytes in general would grow better without it, but as *A. flexile* tends to grow nearer the ground, it may have been more liable to intercept mould spores, and this had an adverse affect of the cultures.

5.4: Growth of the sporophyte

5.4.1 Introduction

Sporophytes were grown from spores collected from a range of field sites, with the intention of comparing the parent morphology with that of the progeny. More than 120 sets of spores from separate parent plants were sown and just over seventy eventually provided sporophytes. These were grown for different purposes. Every batch of spores was grown in a separate pot to produce sporophytes derived from single parent plants. Mature sporophytes were used for the desiccation and freezing experiments already described, (3.4.3 & 3.4.4), and others were grown with different levels of nutrient.

There was very little contamination by other species and it soon became apparent that some *A. distentifolium* plants had produced gametophytes that grew into either *A. flexile* or *A. distentifolium* sporophytes while all the *A. flexile* collections of spores produced only *A. flexile* sporophytes. This was one of the most significant findings in the whole project. The implication, developed in 5.6, is of a genetic combination that produces the *A. flexile* taxon.

Fern gametophytes have three methods of fertilisation of a gametophyte to produce a sporophyte. A single gametophyte can produce male gametes that could fertilise the female archegonia. Since the gametophyte is haploid this means that the sporophyte will be homozygous for all loci, and all sporophytes produced by that gametophyte will be genetically identical. Gametophytes from the same parent plant could cross-fertilise giving wider genetic variation, but this is still inbreeding. The third option is of fertilisation between two gametophytes from different parent plants that is true out-crossing. To further examine the production of two taxa from some *A. distentifolium* plants, gametophytes were isolated so that they self-fertilised, or they were combined with gametophytes of either *A. flexile* and *A. distentifolium* from known sources to investigate their interaction and the resulting sporophyte. There is a more detailed consideration of the factors regulating breeding systems in 6.2.

An experiment that was continued for two years was designed to contrast the growth and fertility of sporophytes that were chilled in the winter, with others which remained in the cold frame. Hill (1976) found that ferns were more fertile after a longer period of chilling, and it was expected that something similar would result. This first growth experiment had a low-nutrient compost.

Although it had been found that the two taxa could be derived from the same parent, they showed different growth responses. As a progression from the observations made on gametophyte growth, sporophytes of both taxa were grown to assess their response to different levels of nutrients. In this second growth experiment, attention was focused around the frond height and spore production.

5.4.2: Materials and methods

a) Source of the gametophytes

Abundant supplies of gametophytes from the same parent plant were available growing on the gel medium from earlier germination experiments. These were lifted off with a needle and placed onto surface-sterilised compost in pots, instead of sowing fresh spores on the compost.

Gametophytes to be isolated or placed together in pairs, were derived from specially germinated spores, grown as usual on a pH 3.8 medium (5.3.2) and separated when one week old. (Appendix 2 for sources used)

b) The growing medium

Gametophytes that were isolated, or paired in single cells were placed in dishes with 5 x 5 cells with the standard pH 3.8 medium.

The compost for the cultivation of gametophytes was a standard mix of one part grit to five parts composted bark with four granules of slow-release fertiliser, Osmocote, (N:P:K, 14:13:13) in an eight centimetre square pot. The loosely compacted compost in the pots was surface sterilised by pouring boiling water over the compost that was

covered in cling film until cool. The compost mixture for mature sporophytes was five parts composted bark to one part grit with 5 grams of Osmocote in ten litres.

The first growth experiment was set up in April 1995 and sporophytes were initially grown in the cold frame, using a commercial compost mix intended for seed germination. This consisted of peat, composted bark and grit with some fertiliser included.

In the second growth experiment, one year old sporophytes with most of the compost shaken off the roots were given one of three nutrient levels in the 5:1 composted bark/grit mixture. They either had no nutrient, five grams of Osmocote slow-release granular fertiliser, or ten grams of fertiliser, each in a standard plant tray.

c) Culture of the gametophytes

One week old gametophytes were isolated in individual cells to monitor the number of successful sporophytes produced by self-fertilisation. Other gametophytes, *A. flexile* and *A. distentifolium,* were placed in pairs, the *A. flexile* gametophyte always on the right, to compare the number of sporophytes produced by outcrossing with the number produced by self-fertilisation. These were kept in three incubators at 15 °C and rotated between shelves and incubators each week. They were examined at weekly intervals and the appearance of a sporophyte recorded. Periodically the sporophytes were removed into small pots of standard compost and eventually grown in the glasshouse.

The gametophytes on compost were grown in an incubator with 24 hour daylength 135 μmol m^{-2} s^{-1} at approximately 15 °C. The pots were completely enclosed in three layers of cling film. At intervals, the cling film was removed, the gametophytes watered with distilled water, and then wrapped up again in cling film. The pots were deliberately under filled to allow at least 2 cm for the growth of the first fronds.

d) Culture of the sporophytes

As sporophytes were produced they were gently removed from the gametophytes with forceps and placed into pots or trays. Initially they were wrapped in cling film and kept on a north-facing window sill in the lab until good growth was established. Once the new fronds were pressing against the cling film, it was perforated, and eventually removed. When growing strongly, the plants were placed on the glasshouse bench in the winter, or the cold frame in the summer. In the winter, the plants received 18 hours illumination. All year round the glasshouse was heated to a minimum of 20° C. While some plants were grown in individual pots, the more successful were grown in trays which were less likely to suffer from uneven watering. The cold frame, which was north-facing, had white shading sprayed on the glass in the summer months. The young glasshouse plants were kept under green net shading that cut out 35 % of the light.

In the first growth experiment, the plants were grown for two years, being repotted once. Two groups of 20 *A. distentifolium* and 20 *A. flexile* were grown from April 1995 to June 1997, either in the cold frame for one group, or the cold frame with five months from November to April in the cold room at 4° C for the other group. The cold room plants were wrapped in cling film to prevent desiccation. When finally scored, the length of each frond was measured and the percentage fertility recorded.

In the second growth experiment, one tray each of eight one-year old plants of *A. distentifolium* and *A. flexile* were grown at the three levels of nutrient. They were established at the beginning of June 1997, kept in the glasshouse at around 20 °C with the trays rotated every four weeks. They were scored as above after 16 weeks.

Many more gametophytes were cultured than developed into sporophytes. Some were slow to grow as gametophytes, others produced sporophytes that then died. It had been intended to grow fifty of each batch of sporophytes but there was a high mortality rate at the early glasshouse stage when the sporelings suffered from uneven watering and fluctuating temperatures.

5.4.3:Results

a) Sporophytes

Spores from 71 different wild sporophytes from nine different sites were successfully grown to maturity (Table 5.4.1).

Table 5.4.1 Number of plants from different sources that provided spores that were successfully grown to obtain sporophytes. *F* = *A. flexile, D* = *A. distentifolium*

	1994		1995		1996	
	F	*D*	*F*	*D*	*F*	*D*
Ben Alder	1		8	5		
Bidean nam Bian		1				
Beinn Achaladair				1		
Beinn Eibhinn					4	7
Beinn a' Chreachain			2			
Bridge of Orchy			8	8	8	7
Creag Meagaidh		1	3			
Glen Doll						1
Glen Prosen			3	1		2

Gametophytes from the monitored plants at Glen Prosen often did not grow well, and few sporophytes were obtained. Spores from many of the Bridge of Orchy monitored plants were grown for two years in succession to confirm the results of the first year. It was found that of the thirty-seven sources of *A. flexile* that were sown nearly all produced *A. flexile* sporophytes (Table 5.4.2). One culture had three *A. distentifolium* sporophytes among twenty-nine *A. flexile* plants from a Bridge of Orchy plant, and another source from Creag Meagaidh had one *A. distentifolium* among twenty-two *A. flexile* plants. These could be the result of contamination. Fourteen *A. distentifolium* sets of spores produced only *A. distentifolium*, but 20 sets of spores had a higher proportion of *A. flexile* present than would be expected from occasional contamination (Table 5.4.2). Two hundred and fifty *A. flexile* plants were produced among 381 *A. distentifolium*.

Table 5.4.2 The progeny of *A. flexile* and *A. distentifolium* from batches of spores with known parentage showing two types of *A. distentifolium*, one that bred true, the other that produced *A. flexile*

Parent type	Number of sowings	*A. flexile* progeny	*A. distentifolium* progeny
A. flexile	37	665	4
A. distentifolium	14		423
A. distentifolium both types	20	250	381

The monitored plants at Bridge of Orchy were all sown in two successive seasons to compare the results (Table 5.4.3). *A. distentifolium* No. 1 and No. 5 only produced *A. distentifolium* in both years and *A. distentifolium* No. 8 might also be the same, for the one *A. flexile* which appeared in 1996 could have been the result of contamination. A larger sample of thirty-five sporophytes of *A. distentifolium* No. 8 in 1997 was pure *A. distentifolium*. All the other monitored *A. distentifolium* at this site produced both *A. distentifolium* and *A. flexile* in one or both of the years.

Table 5.4.3 Monitored plants of *A. distentifolium* at Bridge of Orchy some of which produced two types of sporophyte (shaded) *F* = *A. flexile* *D* = *A. distentifolium*

	Number of sporophytes			
	F 1996	*D* 1996	*F* 1997	*D* 1997
A. distentifolium 1		7		49
A. distentifolium 2	21	32	11	30
A. distentifolium 3	8	10	-	-
A. distentifolium 4	15	35	-	-
A. distentifolium 5		8		24
A. distentifolium 6	16	23	-	-
A. distentifolium 7	7	21	15	20
A. distentifolium 8	1	7		35
A. distentifolium 9	-	-	3	13
A. distentifolium 10	-	-	7	23

A. distentifolium spores from Ben Alder and Beinn Eibhinn also showed this capacity to produce the two types (Table 5.4.4). Two out of five *A. distentifolium* from Ben Alder, and four out of seven plants from Ben Eibhinn displayed this characteristic.

Table 5.4.4 Plants of *A. distentifolium* at Ben Alder and Beinn Eibhinn some of which produced two types of sporophyte *F* = *A. flexile D* = *A. distentifolium*

	Number of sporophytes			
	F	*D*	*F*	*D*
Ben Alder	1996	1996	1997	1997
A. distentifolium 2		15		
A. distentifolium 3		5		
A. distentifolium 4	19	29		
A. distentifolium 6		27		
A. distentifolium 7	5	15		
Beinn Eibhinn				
A. distentifolium 2				67
A. distentifolium 3			10	19
A. distentifolium 4				23
A. distentifolium 5			8	26
A. distentifolium 7				11
A. distentifolium 8			4	21
A. distentifolium 9			18	14

Sets of 100 individual gametophytes isolated in single cells could only produce a gametophyte by self-fertilisation. Pure *A. distentifolium* from Glen Doll produced only *A. distentifolium*. Three sets of *A. flexile* gametophytes produced only *A. flexile*. Two sets of *A. distentifolium* from Bridge of Orchy produced both taxa (Table 5.4.5).

Table 5.4.5 Ratio of plants produced from selfed gametophytes showing that pure strains of *A. distentifolium* and *A. flexile* produce the same type, but some *A. distentifolium* can produce both.

Source	Number of selfings which occurred	Number of surviving sporophytes	
		A. flexile	*A. distentifolium*
Glen Doll *A. distentifolium*	50		44
Bridge of Orchy *A. distentifolium* 2	23	10	11
Bridge of Orchy *A. distentifolium* 7	31	11	9
Ben Alder *A. flexile*	19	16	
Bridge of Orchy *A. flexile*	44	36	
Beinn Eibhinn *A. flexile*	20	20	

When gametophytes of *A. flexile* and *A. distentifolium* were paired together twelve pairs were fertilised. Of these, five of the sporophytes of *A. distentifolium* parents and four from the *A. flexile* parents subsequently died. The seven surviving *A. distentifolium* gametophytes all produced *A. distentifolium* sporophytes. Five of the *A. flexile* gametophytes produced *A. flexile* sporophytes, but three *A. flexile* gametophytes produced *A. distentifolium* sporophytes. This implies that *A. distentifolium* appears to be dominant to *A. flexile* (Table 5.4.6).

Table 5.4.6 Showing the result of cross fertilisation between 12 pairs of gametophytes of *A. distentifolium* (*D*) and *A. flexile* (*F*).

	D	***F***	
Gametophytes	12	12	
Died	5	4	
Remaining	7	8	
	↓	↓ →	**3 *D***
Resulting progeny	7 *D*	5 *F*	

b) Variation in the sporophyte

Individual plants of *A. distentifolium* or *A. flexile* were usually very distinct from one another but there was some morphological variation. A wider range of morphology was found in cultivation than had been observed in the field. The sporophytes grown in the glasshouse and cold frame from three plants of *A. flexile* from Creag Meagaidh and two from Beinn a' Chreachain all had bulbils in the axils of the two lowermost pinnae. These rooted into the compost and some detached bulbils placed in compost grew successfully. A few examples of bulbils were also found on occasional plants of other *A. flexile* in cultivation, but not in the field. This has not been reported before in either *A. flexile* or *A. distentifolium.*

Almost the whole range of morphological variation was seen in the monitored plant *A. distentifolium* No. 7 from Bridge of Orchy. This plant produced both the *A. flexile* and *A. distentifolium* type with some common characteristics that were seen in both.

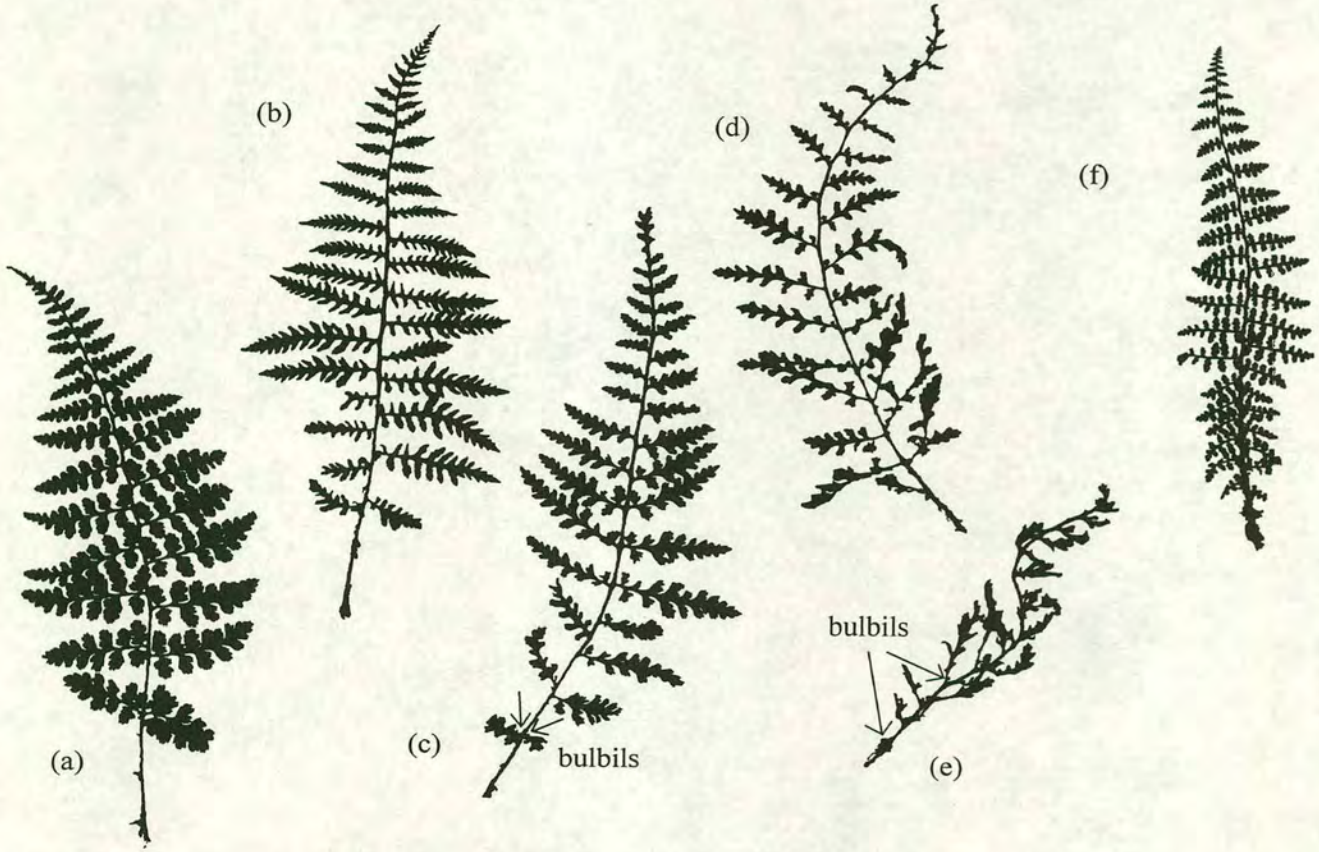
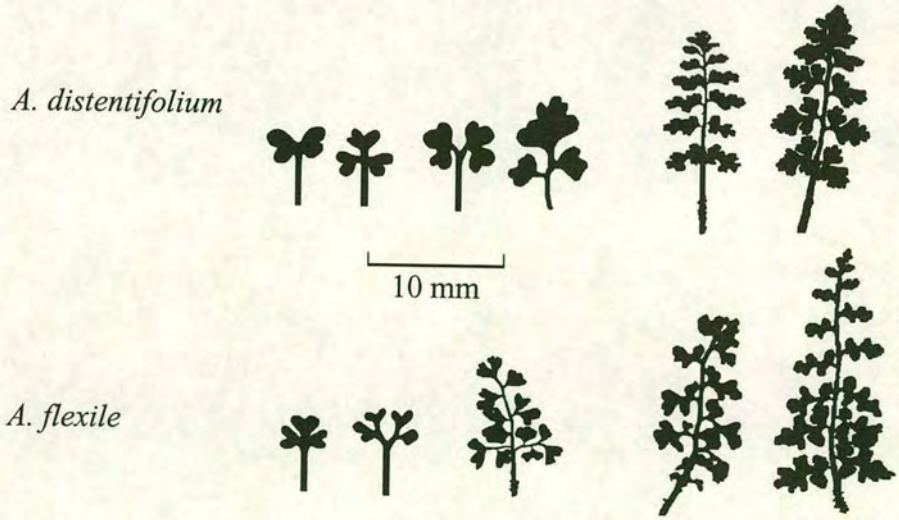


Figure 5.4.1 Fronds taken from one-year-old plants all grown from the same parent plant, an *A. distentifolium* from Bridge of Orchy. (a) is a typical *A. distentifolium* frond, (b) is a narrow-pinna form of *A. distentifolium*, (c) is also narrow fronded with a pair of bulbils in the axils of the lowest pinnae, (d) is a narrow-pinna form of *A. flexile* with some bulbils, (e) is an extreme form of *A. flexile* with bulbils in every axil, (f) is a typical *A. flexile.* The living plants show a marked contrast between the yellow-green of the *A. distentifolium* type, and the blue-green of *A. flexile*.

Occasionally a narrow-pinna form of *A. distentifolium* had occurred as in one set of plants from Ben Alder. In the Bridge of Orchy plants (Figures 5.4.1a-f) that were all derived from the same parent plant, the typical broad pinnules of *A. distentifolium* (Figure 5.4.1a) contrasted with a narrow-pinna form of *A. distentifolium* (Figure 5.4.1b). For the first time one of the *A. distentifolium* plants was observed to have one pair of bulbils in the axils of the lowermost pinnae (Figure 5.4.1c). The narrow-pinna form was also found in the *flexile* type (Figure 5.4.1d). An extreme form of this type had bulbils in the axil of every pinna (Figure 5.4.1e). There was also typical *A. flexile* (Figure 5.4.1f). It was nevertheless still possible to divide the two taxa on the basis of colour; the *A. distentifolium* were more yellow, the *A. flexile* bluer green. The *A. distentifolium* sporophytes also maintained the wider spacing of the lower pinnae.

It was often possible to determine the identity of *A. distentifolium* or *A. flexile* from the very early fronds (Figure 5.4.2).

Figure 5.4.2 Silhouettes taken from different young plants showing the early development of *A. distentifolium* and *A. flexile* from the first fronds.



With continuous cultivation, sporophytes of *A. flexile* produced spores within nine months, although in the field such continuous growth would not be possible.

A. distentifolium, in contrast, only produced a few fertile fronds after twenty months and with a higher level of nutrient provided.

c) Growth at different nutrient levels

In the first growth experiment, half of the plants were placed in the cold room for five months. This group generally performed less well than the others (Table 5.4.6). None of the *A. distentifolium* in either group were fertile, but the cold frame plants of *A. flexile* had a mean of 44% of the fronds fertile and the cold store *A. flexile* plants were significantly different with only half as many fronds fertile (Table 5.4.7). Usually in the field, *A. distentifolium* is two or three times the height of *A. flexile*, but all the cold frame plants were similar in height and the cold store *A. flexile* were taller than the *A. distentifolium* with a mean of more than 8 cm compared with just over 5 cm for the *A. distentifolium*.

Table 5.4.7 First growth experiment. Height and fertility of sporophytes grown in the cold frame on low-nutrient compost compared with sporophytes treated in the same way but which also spent 5 months in the cold store. The fertility percentages for *A. flexile* were significantly different at the 5% level with a Mann Whitney test where U = 191.5 (± standard error).

	A. distentifolium Height (cm)	n	Fertile fronds	*A. flexile* Height (cm)	n	Fertile fronds
Cold frame	8.14 ± 0.34	14	0	7.85 ± 1.04	13	44 % ± 8.1
Cold store	4.61 ± 0.63	15	0	8.10 ± 1.12	16	21 % ± 4.9

In the second growth experiment neither *A. flexile* nor *A. distentifolium* grew well with no nutrient (Table 5.4.8). With five grams of Osmocote, *A. flexile* had a mean height of just over 11 cm and was 44.4 % fertile. *A. distentifolium* was taller at just over 13 cm but was not fertile. Only with ten grams of fertiliser were 10% of the *A. distentifolium* fronds fertile. Growth was also improved with a mean height of 17.7 cm. *A. flexile* was nearly twice as fertile with ten grams of fertiliser, but very little taller. The main difference in *A. flexile* at the highest level of nutrient was an increase in the number of fronds. At five grams *A. flexile* had a mean of just over seven fronds on each plant but at ten grams there were almost twice as many.

A. distentifolium, in comparison showed little variation from 6.3 fronds at the lower level to 7.8 fronds at the higher level.

Table 5.4.8 Second growth experiment. Height, number of fronds and fertility of sporophytes grown in the glasshouse with three levels of nutrient (± standard error).

Level of nutrient	Height (cm.)	Number of fronds	% fronds fertile
10g. *A. flexile*	12.9 ± 0.64	14.2 ± 1.25	78.6 ± 6.9
10g.*A. distentifolium*	17.7 ± 0.86	7.8 ± 0.7	10.4 ± 7.9
5g. *A. flexile*	11.3 ± 0.52	7.4 ± 0.78	44.4 ± 11.92
5g. *A. distentifolium*	13.6 ± 0.77	6.3 ± 0.67	0
0g. *A. flexile*	4.4 ± 0.31	3.0 ± 0.76	0
0g. *A. distentifolium*	4.3 ± 0.37	2.3 ± 0.42	0

d) Senescence

Plants died down with the first frost. If they were kept cool but frost-free with a natural daylength they remained green until December. Plants wilted rapidly if not watered but could recover if only droughted for one day. If they were unwatered for any longer, they died down, but soon flushed a new set of fronds. New growth could be induced by repotting at any time during the growing season, May to September, when the fresh supply of nutrients stimulated growth.

5.4.4: Discussion and Conclusions

One of the most interesting results from cultivating these sporophytes was the discovery that some apparently typical plants of *A. distentifolium* can produce progeny with the morphology of either *A. distentifolium* or *A. flexile*. It was possible that spores from *A. flexile* contaminated some cultures of *A. distentifolium* but this was eliminated by growing spores from the same marked plants at Bridge of Orchy in two successive years when similar results were obtained. Although the Glen Prosen plants were difficult to grow, one plant also demonstrated this over two years. Spores collected from Ben Alder and Beinn Eibhinn showed the two kinds of *A. distentifolium* - some that bred true, and others that produced *A. distentifolium* and *A. flexile*. As *A. flexile* is a smaller plant and less frequent, it would have been less

likely to contaminate *A. distentifolium*. There was very little apparent contamination of the *A. flexile*, and it was always the *A. distentifolium* that could produce either type. The simple explanation is that *A. flexile* is the result of a recessive gene that provides a distinctive morphology. The appearance of three *A. distentifolium* sporophytes from *A. flexile* gametophytes, (Table 5.4.6), indicated that when the opportunity to cross occurred, the *A. distentifolium* morphology was dominant.

The set of characters for *A. flexile* must be strongly linked as the plants in different sites are generally consistent. More variation was found in cultivated sporophytes than in the wild, but extreme forms without specifically advantageous adaptations would have little ability to survive. The shared features of the narrow-pinna form of both *A. flexile* and *A. distentifolium*, (Figure 5.4.1), showed that some morphological characters can cross boundaries between the two main types. However, the linked gene, or set of genes, which make up *A. flexile*'s distinctive morphology also carry a different response to nutrients. This shows that *A. flexile* not only looks different but also behaves in a different way.

When the first growth experiment plants were grown for two years in the cold frame, the plants that were placed in the cold room had a more consistent period of chilling than the ones which remained outside. The cold frame was left open and although it was a mild winter these plants experienced occasional frost, unlike the cold room plants. It is possible that the polythene wrapping to prevent desiccation of the cold store plants may have been more anoxic than would have occurred naturally. Hill (1976) discovered that *Adiantum pedatum* was infertile after only one week of cold treatment, but fertile with a longer period and it is possible that the different amounts of chilling were related to the fertility rates. The statistically significantly higher fertility of the plants that overwintered in the open cold frame, suggested they were in a more suitable environment. The following season's fertility can be determined by nutrition in the previous season (Wardlaw and Sharma, 1963). It is possible to see sporangia immediately the fronds start to expand. These might have aborted under less favourable wintering conditions. It should be possible to examine the next

season's croziers in the autumn to establish potential fertility. An experiment could be devised to establish whether potentially fertile plants can be influenced by their treatment overwinter. Other plants that were kept in continuous growth with an extended daylength were able to produce fertile fronds within a few months of repotting into new fertiliser. This suggests that fertility can be promoted very rapidly with the right conditions.

It has already been found that *A. flexile* gametophytes can grow larger in a low-nutrient environment than *A. distentifolium* gametophytes (5.3), and this more efficient application of limited resources is continued into the growth of *A. flexile* sporophytes. In the second growth experiment *A. flexile* was fertile at a lower level of nutrient than *A. distentifolium* (Table 5.4.9). With more nutrient available, *A. flexile* was more fertile, but instead of using the nutrients to increase height like *A. distentifolium*, more fronds were produced. This implied an upper limit on size and indicated a different use of resources.

The occurrence of bulbils is a not uncommon feature in ferns. A variety of *Polystichum setiferum* (Forsk.) Woynar, native to Britain, has abundant bulbils along the length of the rachis, in the axils of the pinnae. Their appearance in cultivated material of *A. flexile* indicated a latent feature that is not apparently being expressed in wild plants. A long season, with exceptional warmth and humidity might approximate to the glasshouse conditions that promoted these bulbils. The near-prostrate habit of *A. flexile* is more suited to the successful rooting of bulbils than the more vigorous upright fronds of *A. distentifolium*.

5.5: Phenology in the field

5.5.1 Introduction

An important part of the project was to monitor plants growing naturally in field sites. Visits at two-week intervals throughout the growing season enabled measurements to be taken as the fronds expanded, through maturation times for the spores until final senescence. This gave information to compare with cultured plants, and regular visits to the same sites demonstrated the inhospitable environment in which these plants grow. Two seasons' data were gathered. Although the individual seasons were not very similar, the overall timing of expansion was surprisingly so, moving earlier or later according to the season.

5.5.2 Materials and methods

Ten crowns of *A. flexile* and *A. distentifolium* were selected at the two field sites near Bridge of Orchy and in Glen Prosen. The plants were chosen at well-spaced intervals around the populations and at points which could be relocated. As most of the ferns grew in large clumps, the extreme right or left crown was chosen to be the marked plant. A small inconspicuous plastic coated peg with a white label was inserted near the crown. At Glen Prosen all the *A. flexile* grew around the rocks, (see 3.2.1 Figure 3.1), and they were chosen at intervals with three crowns on the east side, two on the north side, one on the less populated west-facing aspect, and four on the south-facing side of the slope, with two under the partially overhanging rock. Only one *A. distentifolium* was chosen from the infertile *A. distentifolium* around the edge, on the north side, the rest were all in the large central clumps, generally distributed.

At Bridge of Orchy, a much larger site, a lump of quartz was placed by the monitored plants to assist in location from a distance, in addition to the peg. Here, there were two marked *A. flexile* and two *A. distentifolium* plants in a scree near the stream on the north-west facing side, four *A. flexile* and three *A. distentifolium* in the first v-shaped scree below a rowan tree, and the remaining plants in the second v-shaped scree (3.2.1, Figure 3.2).

The marked plants were visited at two-weekly intervals throughout the growing season, except for one three-week interval at the beginning of the 1997 season. Once they had started to expand, the length of all the fronds was measured from a point at the base of the crown. As the season progressed, the initiation of a new frond was noted and was included in the mean height of the fronds for that crown if it was taller than 5 cm. Once the fronds were fully expanded, each frond was given a score for the percentage of the frond which was fertile and this gave a mean score for each crown. A pinnule was collected from each plant once the spores appeared to be ripe and this gave a small sample of spores which was sown every two weeks to give a germination percentage. Whole fronds were only collected for spores once full fertility was reached and measuring was concluded for the season. The last visit was in late October or November after the fronds had died down.

5.5.3 Results

a) The initiation of growth

The Bridge of Orchy site was visited from April in 1995, 1996 and 1997. In 1995, there were small amounts of snow in the v-shaped screes but it had melted from this side of the corrie first. Although there was a greater volume of snow on that side of the corrie in 1996, the same scree near the stream came out of the snow before the rest of the population. The ferns mostly grew in the v-shaped screes and these retained the snow. While some ferns were uncovered weeks before others, in 1996, there was little difference in the timing across the population of frond expansion, (which began in the middle of June), and the subsequent maturity of the spores. In 1997 there was no snow on the site in April but the fronds did not start to expand until the end of May (Figures 5.5.1a & b).

The site at Glen Prosen was not found until June 1995 but was visited in April 1996 when there was a large snowbed at least two metres deep down the slope and around the rocks. The rocks themselves were almost free from snow and a deep drift lay all around. Many of the fern crowns were already uncovered. Two weeks later the north

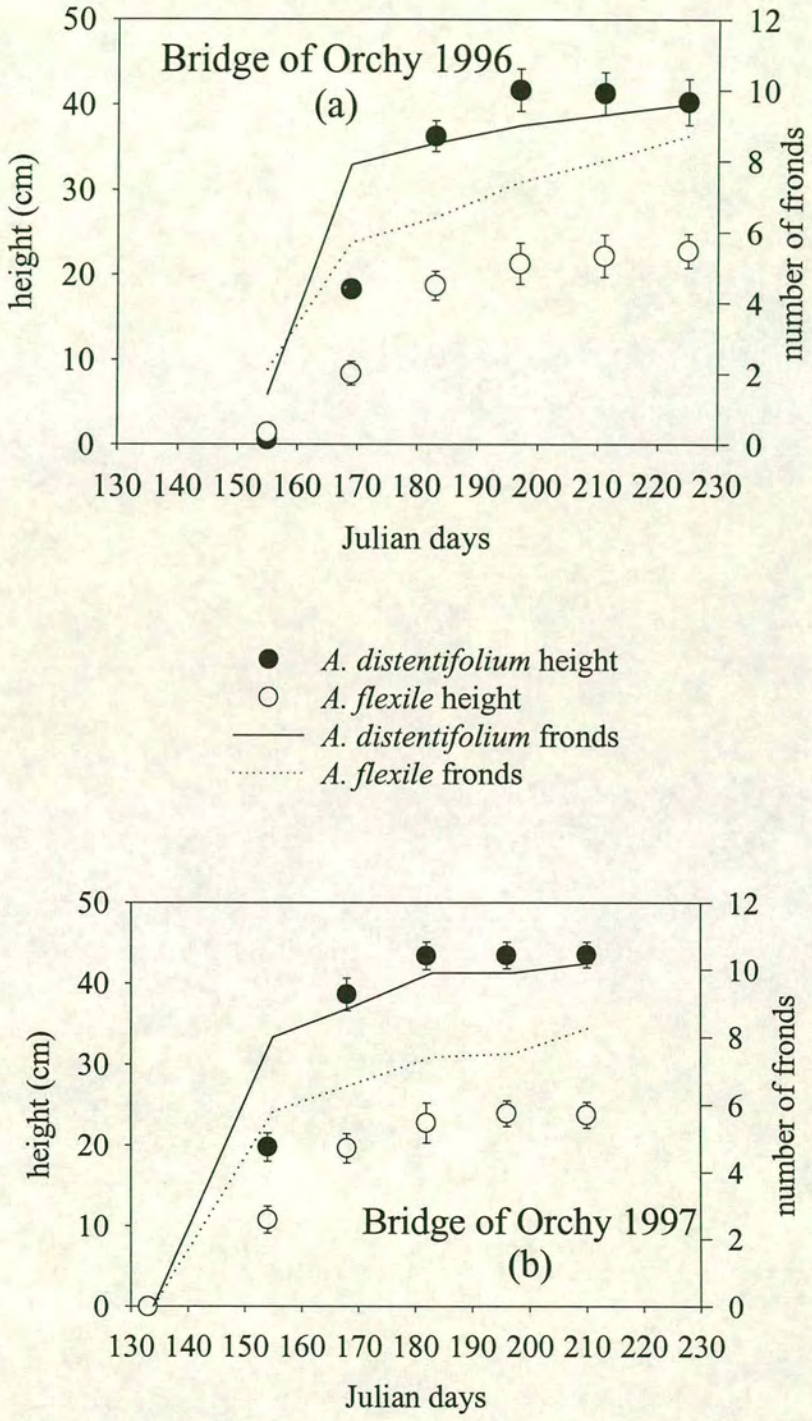
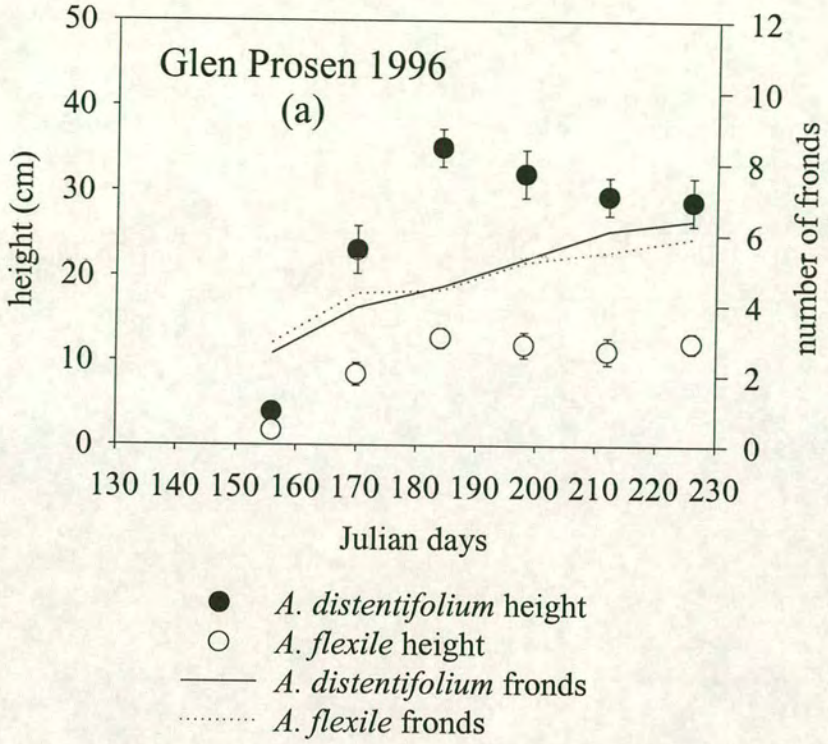


Figure 5.5.1 mean expansion of fronds on marked plants at Bridge of Orchy in 1996 (a) and 1997 (b) together with the mean number of fronds on each plant (standard error bars).



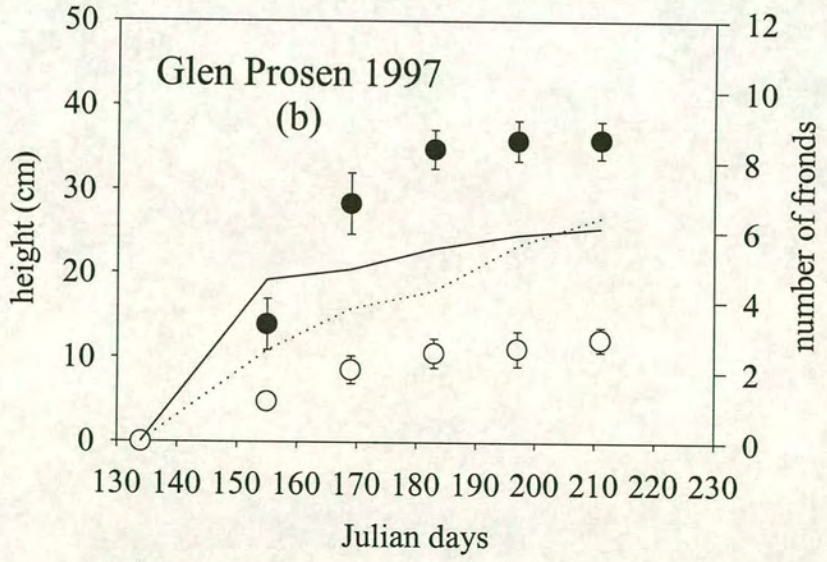


Figure 5.5.2 Mean rate of expansion of fronds on marked plants at Glen Prosen in 1996 (a) and 1997 (b) together with the mean number of fronds on each plant. *A. distentifolium* was grazed in 1996 which reduced the mean height and prompted the production of new fronds (standard error bars).

side of the rocks still had snow one metre deep over some of the ferns, but this had melted in another two weeks. Growth did not begin until June (Figure 5.5.2a). In 1997 there was no snow in April and the ferns started to grow at the beginning of May. A frost before May the 12th left many blackened croziers. Growth was well started again by the beginning of June (Figure 5.5.2b) and the overall level of frond production and fertility seemed little different from the year before. By eleven weeks from expansion *A. distentifolium* had produced 6.1 (SE 0.59) fronds and *A. flexile* 6.4 (SE 0.69) fronds, compared with 5.9 (SE 0.23) and 6.4 (SE 0.76) respectively the previous year. One particular plant which had had six fronds in 1996 had three frosted and only produced three in a second flush in 1997, but this was unusual.

b) Frond expansion

From the first expanding of the croziers until the fronds were fully extended was approximately six or seven weeks (Figures 5.5.1 & 5.5.2). At Bridge of Orchy (Figures 5.5.1a & b) most of the season's fronds started to expand in the first two or three weeks and there was only a gradual addition of new fronds thereafter. *A. flexile* attained up to half the height of *A. distentifolium* and the Bridge of Orchy plants were larger than in other populations with each crown producing more fronds. In 1996, *A. distentifolium* at ten weeks had 9.6 (SE 0.8) fronds while *A. flexile* had 8.7 (SE 0.6) fronds. In 1997 at eleven weeks there were 10.2 (SE 0.95) fronds and 8.3 (SE 0.62) fronds respectively. The maximum mean growth rate of *A. distentifolium* at Bridge of Orchy in 1996 was 18 cm in 14 days during June and July. The *A. flexile* plants had a mean growth rate of 10 cm over the same period.

The Glen Prosen *A. distentifolium* in 1996 had been grazed, and responded by producing new fronds after the main phase of expansion. This distorted the means of the height measurements (Figure 5.5.2a). In 1997 they grew well and produced few new fronds mid-season (Figure 5.5.2b). The *A. flexile* plants continued to add new fronds throughout the season, a trend that is seen more clearly here than at Bridge of Orchy. The mean maximum rate of expansion at the end of June and July in 1996 was 19.2 cm in 14 days for *A. distentifolium* and 6.7 cm in 14 days for *A. flexile*.

c) Spore shedding

At Glen Prosen in 1996 the first spores were ripe ten weeks after the fronds started to expand (Table 5.5.1). Eight out of the ten *A. flexile* monitored plants were fertile and five were shedding by this date with a mean germination percentage of 62%. The germination percentage rose to 83% after two weeks by which time all eight were releasing spores. This level of potential germination was maintained and dead fronds collected at the end of October still yielded viable spores with a mean germination of 84%.

Only five out of the ten monitored *A. distentifolium* plants at Glen Prosen were fertile and four had shed spores by ten weeks (Table 5.5.1). Their overall germination percentage initially was lower, 31%, but within two weeks all five were shedding with good germination: 97%. This declined two weeks later to 85% and the end of season fronds mostly contained spores which had not matured and only gave 3% germination from three of the plants.

Table 5.5.1 Spores collected from the monitored plants which were fertile at Glen Prosen in 1996 and sown to assess their viability. They show a seasonal rise and decline in germination potential for *A. distentifolium*

A. flexile	28.7.96	15.8.96	29.8.96	25.10.96
Total plants fertile	8	8	8	8
Number of plants shedding	5	8	6	6
Range % germination	34-91	50-97	60-99	75-93
Mean % germination	62%	83%	82%	84%
SE	10.4	6.9	6.1	5.1
A. distentifolium				
Total plants fertile	5	5	5	5
Number of plants shedding	4	5	3	3
Range % germination	10-90	90 -99	77-92	2-5
Mean % germination	31%	97%	85%	3%
SE	19.9	1.8	5.4	1

The monitored plants at Bridge of Orchy (Table 5.5.2) showed a similar time-scale in 1996, although the first spores were able to germinate from one *A. flexile* plant in

only eight weeks with 28% germination. Fifty-four percent of the spores from nine plants germinated by ten weeks but it was not until twelve weeks that all the *A. flexile* plants released spores with a 94% mean germination. *A. distentifolium* was similar, rising from 62% at ten weeks to 93% at twelve weeks and the dead fronds produced spores with a mean germination percentage of 92% at the end of October.

Table 5.5.2 Spores collected from monitored plants at Bridge of Orchy which were fertile in 1996 and sown to assess their viability. They show a seasonal increase and the retention by dead fronds of spores with a high germination potential

A. flexile	16.7.96	29.7.96	18.8.96	28.10.96
Total plants fertile	10	10	10	10
Number of plants shedding	1	9	10	8
Range % germination		20-82	70-99	80-99
Mean % germination	28%	54%	94%	95%
SE		6.7	2.8	2.4
A. distentifolium				
Total plants fertile	9	9	9	9
Number of plants shedding	0	9	9	9
Range % germination		4-84	66-99	90-98
Mean % germination		62%	93%	92%
SE		8.7	5.5	0.9

A shorter series of spores was collected from the monitored plants at Bridge of Orchy in 1997 (Table 5.5.3). Four *A. flexile* plants started to release viable spores after only seven weeks with 39% mean germination. By nine weeks, eight of the plants were producing spores with 69% germination and the previous year's maximum was approached by eleven weeks with 89% mean germination. *A. distentifolium* produced viable spores by nine weeks with 56% mean germination and was nearly at a maximum by eleven weeks with 91% mean germination.

Table 5.5.3 Spores collected from monitored plants at Bridge of Orchy which were fertile in 1997 and sown to assess their viability, showing the earlier maturation of *A. flexile* spores

A. flexile	1.7.97	15.7.97	29.7.97
Total plants fertile	10	10	10
Number of plants shedding	4	8	10
Range % germination	14-58	31-94	45-99
Mean % germination	39%	69%	89%
SE	9.1	8.6	5.1
A. distentifolium			
Total plants fertile	9	9	9
Number of plants shedding	0	8	9
Range % germination		32-79	76-95
Mean % germination		56%	91%
SE		5.2	2.1

d) Fertility

Some of the monitored plants did not produced any sporangia. One of the ten monitored *A. distentifolium* at Bridge of Orchy had been fertile in 1995, but was not fertile in either 1996 or 1997. Otherwise the Bridge of Orchy *Athyrium* were more fertile than at other localities that were visited. At Ben Alder and Beinn Eibhinn *A. flexile* was usually observed to be at least partially fertile but *A. distentifolium* frequently was not fertile at all. Not all the *A. flexile* plants at Glen Prosen were fertile; eight were fertile in 1996 and seven in 1997. Five Glen Prosen *A. distentifolium* were fertile in 1996 and seven in 1997.

If the plants were fertile, the sporangia might only be on part of one frond and the percentage fertility given to each plant reflected the number of fronds and the area of each frond which bore sporangia. A greater percentage of the fronds were fertile at Bridge of Orchy in 1997, than 1996 (Figure 5.5.3). *A. distentifolium* appeared to be more fertile in 1997 at Glen Prosen but the scoring in 1996 was affected by grazing before an estimate had been made. New fronds which were produced to compensate for the eaten fronds were not fertile.

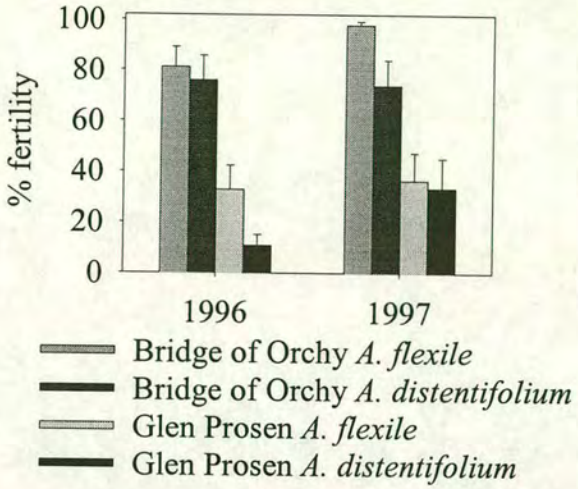


Figure 5.5.3 The percentages of fronds fertile in 1996 and 1997 at Bridge of Orchy and Glen Prosen. *A. flexile* at Bridge of Orchy in 1997 was relatively more fertile than in 1996 while *A. distentifolium* was similar in both years. *A. distentifolium* at Glen Prosen in 1997 appeared to be more fertile only because it had not been eaten.

e) Height and fertility

With widely varying percentages of fertility and some plants being completely infertile, the non-parametric Kendall's rank correlation was used to test for a correlation between the height and fertility of the monitored plants (Table 5.5.4). In 1996 the *A. flexile* plants at both sites showed a positive correlation, although not significant, compared with *A. distentifolium* with almost no correlation. In 1997, the *A. distentifolium* showed a trend towards positive correlation and the Glen Prosen *A. flexile* showed a statistically significant correlation. The Bridge of Orchy *A. flexile* was nearly all 100% fertile regardless of size and did not show a correlation.

Table 5.5.4 Kendall's rank correlation coefficients τ between height and fertility of the ten monitored plants of *A. distentifolium* (*D*) and *A. flexile* (*F*) at the two field sites. (The value of 0.5 must be exceeded for the correlation to be significant at the 5 % level)

	1996		1997	
	D	***F***	***D***	***F***
Glen Prosen	0.1	0.4	0.4	0.66
Bridge of Orchy	0.1	0.42	0.3	0.09

The mean height of the monitored plants (Table 5.5.5) showed only a slight increase in the mean height of *A. distentifolium* between 1996 and 1997 at both sites, and very little difference in *A. flexile*. The difference in height between the two seasons was not statistically significant

Table 5.5.5 Mean height (cm) of fronds of *A. distentifolium* (*D*) and *A. flexile* (*F*) on 10 monitored clumps at two field sites in 1996 and 1997 (SE = standard error).

	1996				1997			
	D	SE	***F***	SE	***D***	SE	***F***	SE
Glen Prosen	29.6	5.5	12.8	1.4	36.0	8.5	12.2	1.0
Bridge of Orchy	40.3	3.3	22.9	1.8	43.6	3.3	23.5	1.9

f) Production of new fronds during the season

New fronds were produced at any point in the season, even in late summer. Damaged plants produced fronds to replace the previous ones but these were not necessarily fertile. The fronds which were produced very late were sometimes frosted before they had fully expanded. Some *A. flexile* fronds did not develop their maximum length but produced fertile spores on foreshortened fronds (Figure 5.5.4). Some fronds were still slightly green in October but they had usually died down by this time.

5 cm

Figure 5.5.4 Late-season frond of *A. flexile* from Bridge of Orchy that had only partially expanded. Collected in September when mature spores were being shed.

5.5.4 Discussion and Conclusions

A clump of *A. distentifolium* required six or seven weeks to fully expand and the first fronds of *A. flexile* followed a similar time-scale. In Norway, Odland (1991) measured individual marked fronds and found that they were completely expanded within 24-27 days which is comparatively faster than the Scottish observations. The growth measurements from the Scottish field sites are an amalgamation of all the fronds in each clump and new, smaller fronds reduced the overall average. The major part of the expansion had occurred by four weeks, but Odland's specimens might have had more consistent, higher, continental temperatures. He gave a mean July temperature at an *A. distentifolium* site at 500 m as 12 °C, when the Glen Prosen mean was nearer 10 °C, more typical of our variable, oceanic climate. In Norway, where the snow could last until August, Odland (1995) found that *A. distentifolium* at 750 m usually commenced growth in June, which did correspond well to the Scottish season. In Central Europe, *A. distentifolium* in the areas of longest snowcover, did not normally emerge until the end of June and commenced growth soon after (Schaminée *et al*, 1992).

The ability to produce new fronds throughout the season indicated that these taxa are not necessarily influenced by daylength. It is assumed that a period of dormancy is induced by falling temperatures. Hill (1976) established that *Athyrium filix-femina* required at least 30 days outside cold treatment to break dormancy before new fronds were produced in response to higher temperature. Hill found that the longer the cold treatment, the sooner growth restarted. If a fern only received a short cold treatment, a long period was required thereafter until growth began again. In April 1997 the Bridge of Orchy ferns were already free from snow, but did not flush until late May This would protect against renewed growth if a mild period occurred mid-winter. The ferns at Bridge of Orchy which were covered by snow in the spring of 1996 emerged at different times, and yet there was little difference across the population in their final growth and maturity. The last to emerge started to flush their fronds very quickly, while the earlier ones had delayed for some time after being free from snow. This might be similar to the response Hill (1976) reported to a short period of

chilling, which delayed the eventual growth. Schaminée *et al.* (1992) found swift frond expansion from late emerging flowering plants, and this would agree with Hill's rapid flushing after a longer cold period. Billings and Bliss (1961) found that flowering plants that were late in being released from the snow, grew comparatively more rapidly and reached their maximum, although smaller, biomass sooner. The overall height of the monitored plants was similar in both seasons, but the *A. distentifolium* at both sites was slightly taller in 1997, with a longer season (Figure 5.5.5). *A. flexile* was almost the same in either season, but with a smaller ultimate height, this taxon might be less affected by length of season.

Some alpine plants like *Ranunculus adoneus* grow through thin snow cover at near-zero temperatures, (Mullen and Schmidt, 1993), but this growth has not been observed at any of the *A. distentifolium* sites. Odland (1995) found that almost no fronds of *A. distentifolium* were produced at soil temperatures below 7 °C and soil temperature was more important than air temperature. As the soil would not warm up very rapidly this would be a check on frond expansion in a brief milder period.

The rapid early summer growth would make heavy demands on stored carbohydrate. Mooney and Billings (1960) found that within one week of snowmelt a *Polygonum* had used 50% of the energy stored in the roots. This was soon replenished and peaked again by autumn. With such a short season, growth is necessarily rapid and energy reserves are used at a fast rate.

The time of spore-shedding is determined by the season. The spores of American *A. distentifolium* were ripe in July or August (Davis, 1965), which corresponded to this country. Sato and Saki (1981a) reported that the spore dispersal period of *A. distentifolium* in northern Japan was only in the first part of September which suggested late emergence and a short growing season. In very late snow beds the fronds were not fertile (Odland, 1991). Presumably too short a season does not allow sufficient reserves to be built up for the production of sori. Wardlaw and Sharma (1963) experimented with *Dryopteris dilatata* plants and found the nutrient

availability the previous year determined the following year's fertility. Their work also helped to explain why replacement fronds were not fertile as they found that defoliated plants produced new fronds, but sori were not present as they would normally have developed during the previous year. The Glen Prosen plants which were eaten were able to produce some fertile fronds in the following year and must have had sufficient time and resources to produce sporangia.

Spore-release at Glen Prosen followed two patterns. The *A. flexile* continued to expand throughout the season and still had spores available to shed after the first frosts. The *A. distentifolium*, with a smaller area of the frond producing spores, shed them earlier and had few left on the dead fronds. At Bridge of Orchy, both taxa produced large numbers of spores late in the season and both were equally able to yield spores from decaying fronds. There was an indication that *A. flexile* can shed spores earlier than *A. distentifolium*. Such an advanced start to the season could allow substantial gametophyte growth in the same year, to *A. flexile*'s advantage, especially if larger competitive gametophytes are produced (5.3).

Odland (1995) found a positive correlation between frond size and fertility and this was demonstrated to varying degrees at the two field sites (Table 5.5.4). The inconsistent results might be related to the length of season, or the plants' response to the previous season. The two seasons were very different, 1996 was late, 1997 was early. The relatively higher correlation of the *A. flexile* plants at both Glen Prosen and Bridge of Orchy in 1996, might imply that *A. flexile* responded to a long winter by comparatively higher fertility relative to the length of frond. *A. distentifolium*, in contrast, might perform better with a longer season like 1997, although both taxa were more fertile in this earlier season.

The ability to produce shortened fronds (Figure 5.5.4) is a useful adaptation enabling the development of the fertile areas without expending reserves on frond extension. Stunted *A. distentifolium* fronds were also produced but did not develop fruitfully as the sporangia were congregated near the tip of the frond and these were not

sufficiently mature. This demonstrated an advantage for *A. flexile* in the positioning of sori near the base of the frond, and helped to explain why some *A. flexile* plants produced mature spores ahead of *A. distentifolium.* The heavily grazed *A. distentifolium* at Glen Prosen in 1996 lost many of the terminal sporangia, a hazard that would not have affected the basal sporangia of *A. flexile.*

There were many stages of maturity observed in sporangia on any one *A. flexile* frond. This gave the capacity to keep growing until finally frosted. The Glen Prosen *A. flexile* plants were examples of this, with the continuous production of new fronds, and variable states of maturity along the length of the narrow fronds. The *A. distentifolium* fronds tend to mature more simultaneously.

5.6: Discussion and conclusions

Comparisons between *A. flexile* and *A. distentifolium* showed that there appeared to be no difference between the taxa in their germination responses to various environmental conditions. A recurring feature was the variation in the response of spores of both taxa from different localities. Ben Alder and Beinn Eibhinn are the two highest sites, well over 850 m, and the spores from these populations had slightly delayed germination and were less able to benefit from the higher temperatures. Populations from different localities appear to be adapted to very local conditions.

The temperatures recorded on the data-logger at Glen Prosen (Figure 3.3) showed higher temperatures in the early part of the season until the end of July, and lower temperatures thereafter. This would provide a high initial temperature for germination, but lower temperatures for the sustained growth of the gametophytes. It was found by experiment, (5.3), that although spores germinated at higher temperatures, the same high temperatures were not suitable for healthy gametophyte growth. This suggests that germination might most readily occur in the spring or the earlier part of the summer

While spore volume had little effect on the capacity to germinate, it did have a bearing on the gametophytes' growth thereafter. When spores were shed from the end of July, it is possible that gametophytes might attain a reasonable size before the winter. Freezing experiments (3.4.3) showed that some gametophytes could survive temperatures as low -20 °C. Larger gametophytes with thicker tissues might have a better chance of survival, but this would require experimentation. If *A. flexile* gametophytes could grow larger than *A. distentifolium* in a low-nutrient environment, this might also increase the chances of survival in extreme conditions.

With the general slow-release fertiliser used in the second growth experiments it was not possible to determine specifically which nutrients *A. distentifolium* required. Even at the highest level, *A. distentifolium* was still only 10% fertile (Table 5.4.9). Neither *A. distentifolium* nor *A. flexile* gametophytes grew well without magnesium, but calcium most strongly affected *A. distentifolium* gametophyte growth, and this might be one of the important minerals in tall, fertile colonies. The most vigorous plants seen in the field were on basic metamorphosed rocks at Beinn an Dothaidh near Bridge of Orchy. Further cultivation tests could investigate which minerals were the most significant in promoting growth and fertility.

The simplest explanation for the appearance of two morphologically distant taxa from the same parent plant is provided by Mendel's work on dominant and recessive genes (Bateson, 1909). All populations have genetic variation, much of which may not have a physical expression and goes unnoticed. All genera occasionally produce sports or monstrous forms and *Athyrium filix-femina*, for example, has given rise to an exceptionally large number of varieties. These are rarely seen in the wild. When found, they are usually single plants with no selective advantage and are only perpetuated by careful nurture in cultivation. A frequently observed mutation in ferns causes irregularities in the shape of the frond, as can be found in *Phyllitis scolopendrium* (L.) Newm. Lang (1923) discovered that spores from an apparently normal *Phyllitis* produced some sporophytes with incised lobes on the fronds while others were entire like the parent plant. He produced several hundred sporophytes

that gave the ratio of 73% entire fronds: 27% incised. As this population resulting from mixed mating had a smaller proportion of incised fronds this frond characteristic appears to be recessive.

A parallel may be drawn with *A. distentifolium* and *flexile*. It appears there are two types of *A. distentifolium* in the same populations with the same morphology. One is a pure-breeding homozygous form which always produces *A. distentifolium*, and the other a heterozygous form that also has the potential to produce the *flexile* variety. Mendelian principles give the likely ratio of sporophytes derived from random mating as 2 heterozygous *distentifolium*:1 homozygous *distentifolium*:1 homozygous *flexile*, giving an overall ratio of 3:1 *distentifolium*:*flexile*, assuming that *flexile* is recessive. This was supported in the experiment where *A. flexile* gametophytes were paired with homozygous *A. distentifolium* gametophytes. Three *A. flexile* gametophytes produced an *A. distentifolium* sporophyte, although none of the *A. distentifolium* gametophytes produced anything other than *A. distentifolium* (Table 5.4.6). This indicated *A. distentifolium* was dominant.

Lang (1923) suggested that if the spores from heterozygous plants were isolated and grown as self-fertilising gametophytes, half would produce plants with incised fronds and half entire; a 1:1 ratio. He attempted to do this but had an inadequately small sample. The selfing experiment (Table 5.4.5) compared the progeny of homozygous *A. flexile* and homozygous *A. distentifolium* with heterozygous *A. distentifolium*. The homozygous *A. flexile* and *A. distentifolium* bred true. The heterozygous *A. distentifolium* were also a small sample but the surviving sporophytes gave ratios of 10:11 and 11:9, which approximated a 1:1 ratio. An isolated gametophyte can only produce one type, either *A. flexile* or *A. distentifolium*, but with mixed mating the dominant *A. distentifolium* alters the ratio. Some of the sporophyte ratios (Tables 5.4.3 & 5.4.4) derived from mixed mating reflect these expected ratios of 3:1 *distentifolium* : *flexile*, but many have a higher proportion of *A. flexile* than might be expected. This might be explained by the apparently greater vigour of some *A. flexile* gametophytes which could become mature sooner than *A. distentifolium* and increase

the chance of *A. flexile* gametophytes cross-fertilising. The low-nutrient compost in the pots might also have favoured the growth of *A. flexile* gametophytes. The classic Mendelian ratios can only be expected with a very high fertility and low mortality rate, or where the success of the two types is similar.

When was realised that the two taxa occurred together in cultures, it was necessary to separate the young plants, as *A. distentifolium* was usually more vigorous. If separation was delayed, and the nutrients exhausted before *A. flexile* was smothered, *A. flexile* could successfully compete. A series of experiments could be devised using spores from a plant known to be heterozygous. These could be grown at different temperatures and nutrient levels, and might give different ratios of the two taxa.

The phenology section (5.5) emphasised the size difference between the two taxa. With a smaller stature, *A. flexile* reached maturity more rapidly when grown from spores. The results from the 1997 field season also indicated that the spores ripened earlier. If the season was very short, or there was an exceptionally early autumn frost, this might be significant in the successful development of spores for *A. flexile* and not *A. distentifolium*. Some of the monitored *A. flexile* plants made use of the longer season by producing successive new fronds, while *A. distentifolium* was less likely to do so. *A. flexile* appears to be more adaptable to an uncertain season and can make use of an extended season if the conditions are suitable.

In the field, *A. flexile* has grown taller than cultivated plants, but the largest plants were always smaller than the largest *A. distentifolium*. In competition with *A. distentifolium*, *A. flexile* would always be smaller and more likely to be shaded. *A. flexile* is only found in sites where the competition from *A. distentifolium* is reduced, as some populations of *A. distentifolium* have tall, dense plants up to one metre high. None of the spores collected from tall *A. distentifolium* at Glen Doll, Bidean nam Bian, Creag Meagaidh or Beinn Achaladair produced anything other than *A. distentifolium*.

CHAPTER SIX Breeding systems and population structure

6.1 Introduction

The suggestion that *A. flexile* is a homozygous recessive, has implications at the time of fertilisation. To maintain *A. flexile* plants within a population, gametophytes either have to self-fertilise or cross with another *A. flexile* gametophyte. It has already been suggested that *A. flexile* responds better than *A. distentifolium* to a nutrient poor environment (5.3 & 5.4), and the gametophytes with *A. flexile* potential could simply grow larger and mature faster to produce a sporophyte in advance of *A. distentifolium*. Many factors can influence fertilisation and the first part of this chapter examines the interaction between gametophytes and the breeding systems that may be involved. Observations on the breeding system have been made on plants growing in the laboratory and glasshouse. Additional observations on the breeding system of wild populations were made through isozyme analysis. Different taxa have different forms of enzymes and a quantification of this difference can often guide taxonomic divisions. Information was sought on the extent of outbreeding in wild plants and the extent to which genetic material was being exchanged between populations.

6.2 Breeding systems

6.2.1 Introduction

Fern gametophytes can either self-fertilise, (intragametophytic selfing), cross between gametophytes from the same parent, (intergametophytic selfing), or outcross between gametophytes from two different parents (intergametophytic crossing). To achieve the *A. flexile* combination there must be fertilisation between two gametophytes with the recessive gene, or intragametophytic selfing of a gametophyte that has the "*flexile* gene". If an *A. flexile* gametophyte is fertilised by an *A. distentifolium* male gamete, the recessive gene would still be present in the population in a heterozygous individual with the *A. distentifolium* morphology. As mature sporophytes appear to be very old, heterozygous individuals can perpetuate

the potential to produce *A. flexile* for a long time, perhaps until the conditions are again suitable for renewed colonisation.

Many species have lethal recessive alleles that, with the homozygous state, cause abortion after fertilisation, so that even if a sporophyte is produced, it soon dies. The frequency with which this abortion occurs contributes to a measure of the genetic load. If there is a long delay before eventual sporophyte formation this may suggest the operation of "leaky lethals" when there are many fertilisations before a successful zygote is formed (Cousens, 1979). Such gametophytes are likely to have a large number of failed zygotes and short-lived young sporophytes. Even if development does occur, a high number of the sporophytes produced in an inbred population die at an early age and the mature plants are smaller (Schneller, 1987). Partly because of this genetic load, many fern gametophytes only produce successful sporophytes by outcrossing (Soltis and Soltis, 1992). If the *A. flexile* gametophytes outcross, this may increase the opportunity of crossing with an *A. distentifolium* gametophyte.

Gametophytes can go through a regular sequence and change gender as they grow. Small gametophytes may be male, then become female as they become larger, with the eventual possibility of being bisexual. This sequence can be affected by interaction with other gametophytes and by nutrition. One mechanism to promote outcrossing is antheridiogen, which is found in mature female gametophytes. In a dense population of gametophytes it can sometimes be observed that there are only a few large prothalli with archegonia and many smaller ones with antheridia. These large female gametophytes have the effect of making other, smaller, presexual gametophytes male, which in turn encourages intergametophytic crossing. Without the presence of antheridiogen, the male prothalli would continue to develop bisexually (Tryon and Vitale, 1977; Schneller, 1979). Different populations and species vary in their genetic load, the amount of inbreeding and their response to antheridiogen. Antheridiogen can be effective up to 10 cm away and as male gametes can swim from 4 to 8 cm this offers a good chance of outcrossing (Schneller *et al*, 1980).

The gametophytes derived from different sized spores indicated that when two gametophytes were growing together, their rate of growth was different when compared with one gametophyte growing on its own (Figure 5.3.4 & 5.3.5). This might be explained by the operation of antheridiogen, where the larger gametophyte could have matured more rapidly and have affected the vigour of the other gametophyte.

To investigate the successful fertilisations from inbreeding and outcrossing gametophytes, gametophytes were isolated or paired to allow either intragametophytic selfing, or intergametophytic crossing. The results gave an indication of the genetic load. Additional information was noted in the variation in the gender of gametophytes grown on the temperature gradient bar.

6.2.2 Materials and methods

Two experiments were set up. Both were in single cells on the Phytagel medium, pH 3.8, using one-week-old gametophytes. The first, with small samples, compared the number of sporophytes produced by intragametophytic selfing, intergametophytic selfing, and intergametophytic crossing. The presence of sporophytes was recorded, but they were not grown to maturity. Functional archegonia and antheridia were observed so that most paired gametophytes had the opportunity to cross. Some gametophytes had numerous archegonia implying failed embryos but these were not examined. Other gametophytes produced many sporophytes on one gametophyte, and these were scored as a single fertilisation (Appendix 4 for spore sources). Many cultures were adversely affected by algae and were discarded, causing uneven sample sizes. Up to seven months were allowed for fertilisation to occur. Some samples were terminated after only four months due to problems with algae.

After the first experiment, it was realised that all the *A. distentifolium* gametophytes that had been used were from heterozygous parents and each had the potential to produce *A. flexile* or *A. distentifolium* sporophytes (5.4.3). This made the

interpretation of the resulting crosses complicated. In the second experiment, there was one batch of *A. distentifolium* spores of known homozygous origin, from Glen Doll, and two batches of heterozygous *A. distentifolium* from the Bridge of Orchy site and three batches of homozygous *A. flexile*. Other batches from further sources had been set up, but many were lost through desiccation and algal growth. In the second experiment, with only intragametophytic selfing, the resulting sporophytes were grown on compost (5.4.2) for six months to assess mortality. Mortality could be due to a genetic defect or horticultural problems.

Gametophytes grown on the temperature gradient bar (5.3) were scored after twelve weeks to assess the presence of antheridia or archegonia at each of the four temperatures, 10, 15, 20, and 25 °C.

6.2.3 Results

a) Selfing and crossing experiments

In the first experiment, there was an indication that gametophytes were more likely to cross fertilise than self-fertilise (Table 6.2.1). Gametophytes of *A. distentifolium* had the lowest percentages of sporophyte production with intragametophytic selfing, higher values for intergametophytic selfing and the highest values for intergametophytic crossing. There appeared to be a difference between *A. distentifolium* and *A. flexile* fertilisation scores, but this might have been due to a particularly high genetic load in the *A. flexile* source, and the *A. distentifolium* gametophytes possibly had more genetic variation as they were all from heterozygous parents. When *A. flexile* gametophytes were placed together from two different parents, or an *A. flexile* gametophyte was placed with *A. distentifolium*, there were still low numbers of sporophytes produced when outcrossing success might have been expected.

Table 6.2.1 Gametophytes grown singly and in pairs, to compare numbers of sporophytes initially produced within seven months by self or cross-fertilisation. *D* = *A. distentifolium*, *F* = *A. flexile*.

	Number		Sporophytes		% fertilised	
	D	*F*	*D*	*F*	*D*	*F*
Intragametophytic selfing	11	35	4	11	**36%**	**31%**
Intergametophytic selfing	22	36	11	7	**50%**	**19%**
Intergametophytic crossing	50	59	39	20	**78%**	**34%**
Intergametophytic crossing *A. distentifolium* x *A flexile*	50		15		**30%**	

With a larger sample in the second experiment (Table 6.2.2) there was considerable variation in the number of fertilisations that occurred - from 19 to 50%. Not all the sporophytes that were fertilised were still alive six months later, but from 16-44% of the original gametophytes had successfully produced a sporophyte. This gave a measure for genetic load for individual plants and showed that self-fertilisation is possible.

Table 6.2.2 One hundred gametophytes from six sources grown in single cells for intragametophytic selfing, showing the number of sporophytes which survived until at least six months after fertilisation. *AD* = *A. distentifolium*, *AF* = *A. flexile*, BO = Bridge of Orchy, BA = Ben Alder, BE = Beinn Eibhinn, GD = Glen Doll.

Source		Successful fertilisations	Number of surviving sporophytes
BAAFG	Homozygous *A. flexile*	19	16
BEAF7	Homozygous *A. flexile*	20	20
BOAF6	Homozygous *A. flexile*	44	36
BOAD2	Heterozygous *A. distentifolium*	23	21
BOAD7	Heterozygous *A. distentifolium*	31	20
GDAD2	Homozygous *A. distentifolium*	50	44

b) Temperature and the production of antheridia and archegonia

Gametophytes grown on the temperature gradient bar showed different gender at varying temperatures. The presence or absence of either gender was noted (Table 6.2.3) but not the proportion. At the highest temperature, 25 °C, all gametophytes that survived and were fertile, had been male since eight weeks. With slower

maturation at 10 °C most gametophytes had only reached the stage of producing antheridia by twelve weeks, except *A. flexile* from Creag Meagaidh, which also had some archegonia. Nearly all the gametophytes had produced either antheridia or archegonia at 15 and 20 °C. The earliest were recorded at six weeks at 20 °C, eight weeks at 15 °C. Moreover, it was observed that many of the stressed gametophytes on the low-nutrient medium (5.3), which were also suffering from low light levels, only produced antheridia (Appendix 5).

Table 6.2.3 Gender of gametophytes grown at different temperatures for 12 weeks that showed only antheridia at 25 and 10 °C and both male and female gametophytes at 15 and 20 °C. M = male, MF = male and/or female, O= neither.

	25 °C	**20 °C**	**15 °C**	**10 °C**
Ben Alder *A. distentifolium*	M	M F	M	M
Ben Alder *A. flexile*	M	M F	M F	M
Ben Alder *A. distentifolium*	M	M F	M F	M
Creag Meagaidh *A. flexile*	M	M F	M F	M F
Creag Meagaidh *A. distentifolium*	O	M F	M F	M
Beinn Eibhinn *A flexile*	M	M F	M F	M
Beinn Eibhinn *A. distentifolium*	M	M F	M F	M
Bridge of Orchy *A. flexile* Y	M	M F	M F	O
Bridge of Orchy *A. distentifolium* '97	M	M F	M F	M
Bridge of Orchy *A. flexile* 5	M	M F	M F	O
Bridge of Orchy *A. distentifolium* '96	M	M F	M F	M
Glen Prosen *A flexile* L	O	M F	M F	O
Glen Doll *A. distentifolium*	O	M F	M F	M
Glen Prosen *A. flexile* 2	M	M	M F	O

6.2.4. Discussion and conclusions

With no genetic load, gametophytes would have nearly 100% success in producing sporophytes. As only up to half of the *A. distentifolium* and *A. flexile* gametophytes were successfully self-fertilised, (Table 6.2.1 and 6.2.2), there may have been lethal alleles present, but the genetic load was not so high that intragametophytic selfing was impossible. The mortality of some of the fertilised sporophytes in the second experiment (Table 6.2.2), indicated that although fertilisation can appear to have been successful, sporophytes do not necessarily survive.

The present populations of *A. distentifolium* and *A. flexile* appear to have been established for a considerable length of time (4.8) with very few young plants. Klekowski (1973) discussed the development of genetic load in a newly established colony of *Osmunda regalis*. The first colonising spores that landed on a site could only survive to produce sporophytes if they had a low genetic load. With increasing sporophytes in the population, more outcrossing would be possible and the genetic load would increase. As the population had a greater opportunity to produce large volumes of spores, this retained the possibility that from the wide genetic variation within the spores, some would have a low genetic load and be able to start new colonies. Genetic load thus tends to be relatively low in colonising species but higher in old established populations. This must have been the case with early colonisation of *A. distentifolium*.

Watano and Masuyama (1991) studied *Ceratopteris thalictroides*, an inbreeding species, and found that only 3 out of 26 populations had genetic load. A lack of a response to antheridiogen was suggested as indicative of no genetic load (Schneller *et al*, 1980) but the antheridiogen mechanism was still in operation with *C. thalictroides* and produced small males with numerous antheridia. It was proposed that the antheridiogen helped to promote outcrossing in an otherwise inbreeding species. To test the effect of antheridiogen in the wild, Hamilton and Lloyd (1991) collected 775 mixed gametophytes of *Deparia acrosticoides* (Sw.) Kato, *Diplazium pycnocarpon* (Spreng.) Brown, *Polystichum acrostichoides* (Michx.) Schott and *Adiantum pedatum* L. in September and October. Of these, 412 were asexual, 300 were male, 50 female and 13 hermaphrodite. Some prothalli grew vigorously, possibly because of better nutrition and became solely female, emitting antheridiogen to make the adjacent prothalli exclusively male. Gametophytes in an intermediate habitat became hermaphrodites. Schneller (1988b) found that wild *Athyrium filix-femina* gametophytes were mostly either male or female with only a few hermaphrodites.

Haufler and Welling (1994) found that antheridiogen can promote dark germination of buried spores. While the prothallus thus produced is not typical, it can have viable antheridia that could fertilise surface prothalli. In this way, buried spores from a different parent sporophyte that could even date back some decades can add to the genetic diversity of the current population. There was no dark germination of *A. flexile* or *A. distentifolium* in six weeks at 15 °C (5.2) but this was not tested in the presence of antheridiogen. Banks *et al*, (1993) found with *Ceratopteris richardii* that antheridiogen from mature gametophytes began to effect the other spores immediately after they had germinated. Antheridiogen must be continually present if the gametophytes were to remain exclusively male.

It has already been demonstrated (5.3) that spore size can affect the size of the resulting gametophyte. Spore size could be significant in the growth of *A. distentifolium* or *A. flexile* as the first spore to germinate has a greater chance of forming a larger, and thus female, gametophyte. While male *A. flexile* gametophytes ensure the continuation of the recessive gene in a population, *A. flexile* sporophytes would only appear in the population if female *A. flexile* gametophytes succeed, and have the chance of crossing with an *A. flexile* male gametophyte. Although the difference in mean spore length between *A. flexile* and *A. distentifolium* is not great, 34.1 μm (SE 0.37) compared with 32.3 μm (SE 0.38), the mode is 36 and 33 μm respectively and *A. flexile* might have a slight initial advantage which could be accentuated by the adaptation to the substrate. The variation in spore size observed in both taxa would also help to promote outcrossing as the gametophytes might attain different sizes even without the effect of antheridiogen. Mottier (1931) reported similar responses from *Matteucia struthiopteris* (L.) Tod. where there was also no clear division into two types of spore. Mottier found that in a mixed culture larger spores produced female gametophytes, and smaller spores produced male gametophytes.

While antheridiogen may have an influence on the interaction between gametophytes, the artificial cultural conditions might also vary the results. The effect

of nutrition was observed in the predominant maleness of the gametophytes grown with low nutrient levels (5.3). Different temperatures also altered the sex ratio (Figure 6.2.3). High temperatures were a source of stress, and only under less stressful conditions are archegonia produced. The presence of female gametophytes in one culture of *A. flexile* at 10 °C could indicate that this taxon can mature faster at lower temperatures and thus gain some advantage over *A. distentifolium*. As this temperature is similar to the mean field growing season temperature (3.2.3) gametophytes could profitably be grown at this low temperature for a longer period as the results might more accurately suggest their response in the wild, than growth at higher temperatures.

Etter (1923) found polyembrony (multiple sporophytes on one gametophyte) developed under experimental conditions with some polypodiaceous ferns, i.e. *Matteucia struthiopteris*, *Onoclea sensibilis*, *Dryopteris mollis* and *Pteris longifolia*. Lang (1923) also observed polyembrony with *Phyllitis scolopendrium*. This was frequently observed for both *A. flexile* and *A. distentifolium*. Up to fourteen sporophytes were observed on one well-grown gametophyte. When grown on together this was a useful check that the gametophyte had self-fertilised as the same-source sporophytes all had similar characteristics, like a narrow-pinna form. It was also observed that even after the sporophytes of both *A. flexile* and *A. distentifolium* had been growing for some months, gametophytes were still present at the base of the sporophyte. If the sporophyte should die, this would allow further fertilisation. Haig and Westoby (1988) suggested that polyembrony was a mechanism that assisted in the selection of fitter embryo genotypes. Only the most vigorous would survive with the immediate competition from other sporophytes on the same gametophyte.

Mottier (1931) maintained well-nourished gametophytes of *Osmunda* and *Matteucia* for eight years without fertilisation occurring. With excessive nutrient they produced branched prothalli, vegetative growth and only archegonia. Antheridia only appeared when the gametophytes were stressed. On the micro-habitat scale small differences of topography could give local variations affecting gametophyte gender, and promoting

outcrossing. For the continued success of the sporophyte, fertilisation ought only to occur in a suitable environment for continued growth. Korpelainen (1994) grew two populations of *Dryopteris filix-mas* on different nutrients and found that the size of the gametophyte, sex, reproduction and mortality varied according to the source and nutrient level. There was a wide variation in the selfing rates of the two populations on different nutrients, ranging from 33-96 % for one population, and 54-100% for the other. There was considerable difference noted between the selfing and crossing rates observed for gametophytes of *A. distentifolium* and *A. flexile* (Table 6.2.2) both within and between taxa. Some gametophytes grew vigorously and produced sporophytes, others grew vigorously and produced very few sporophytes, while others grew slowly. The sixteen sets of gametophytes grown on different nutrients (Figure 5.2.6) indicated that even from the same locality, the spores from different individuals can produce batches of gametophytes that seem to have varying preferences. This wide variation in habitat preference, however, does increase the chance that of the many million of spores produced, some might land somewhere appropriate.

6.3: Population structure

6.3.1: Introduction

Isozyme analysis can give insights on a molecular level into large-scale patterns in wild populations. Enzymes can be compared to make inferences about the exchange of genetic material between and within populations. Banding patterns are produced as different enzymes are stained after electrophoretic separation on gels, and these may show heterozygosity in a population (Haufler and Soltis, 1984) and indicate outbreeding. The occurrence of intragametophytic selfing can be inferred from the frequency of homozygosity at variable polymorphic loci (Ritland *et al*, 1990). A measure of genetic variation is the proportion of loci that are polymorphic, and the frequency of alleles per locus in a population can be compared statistically (Soltis and Soltis, 1990) to give comparisons between populations.

A pilot study in 1995 used two cultivated samples each of *A. distentifolium* and *A. flexile* from Ben Alder, Glen Prosen, Creag Meagaidh and Bridge of Orchy. There was also *A. distentifolium* from Caenlochan and *A. filix-femina* from the garden. A phosphate grinding buffer (Soltis *et al*, 1983) was used and four enzymes were stained, Aspartate aminotransferase (AAT), 6-Phospho-D-gluconate (6PGD), Phosphoglucomutase, (PGM) and Phosphoglucoisomerase (PGI). These showed that while *A. filix-femina* had distinctly different banding, there were no apparent differences between *A. flexile* and *A. distentifolium*. It was noted that there were small differences between populations. Johannes Vogel in the Conservation Biology Laboratory at the Natural History Museum in London offered to collaborate in the analysis of a larger number of samples with a wider range of enzymes. Accordingly, fronds were collected from all the known sites of *A. flexile* and sent to London to be processed with *A. distentifolium* from the same sites and elsewhere in Scotland and three sites in Europe. A full account will be published jointly, and a summary of the findings is included here.

6.3.2: Materials and methods

Fronds were collected in the field in July and August 1997 from the six populations where *A. flexile* has been found during this study, and from three populations of *A. distentifolium* where *A. flexile* has not been found. Fifty fronds of *A. flexile* and 50 fronds of *A. distentifolium* were collected from Ben Alder and Bridge of Orchy, the two largest sites. Up to 30 fronds were collected of each taxon from the other sites. Fronds of *A. distentifolium* were also collected from Ben Wyvis, above Loch a' Mhadaidh and Craig an Duine by Clive Jermy, from Switzerland by Jakob Schneller and the Pyrenees and Germany by Stefan Jessen. More than 600 samples were gathered (Table 6.3.1).

At the location where the samples were to be collected, distinct sub-populations were identified within the overall range of the plant, usually as separate areas of scree. Samples were selected to include peripheral sub-populations and single plants. An indication of distance apart was noted in the field. At most sites the sampling points were 50 to 100 metres apart. As Glen Prosen was such a small site the plants were as little as one metre apart. Four or five samples were collected from each sampling point, up to ten samples from the larger areas with no obvious discontinuity. A whole frond was collected from small plants, but only the upper 15 cm was taken from large fronds. Fully expanded infertile material was preferred. Fronds were collected from all the monitored plants (5.5) and separately labelled. The sources of all the plants collected for the study are shown in Table 6.3.1.

The fronds were posted to the Natural History Museum in London the day after they were collected. On arrival they were ground and stored at -80° C. Methods used at the Natural History Museum are in Appendix 6 together with a preliminary report by Johannes Vogel summarising his analysis. The gels were scored by Johannes Vogel and Fred Rumsey with reference to their previous experience, particularly with *Asplenium*.

Table 6.3.1 Sources of specimens of collected in 1997 for isozyme analysis

Code	Taxon	Source	Collector	n
Alps	*A. distentifolium*	Alps, Val di Piorina, Southern Switzerland	J. J. Schneller	30
Erzgebirge	*A. distentifolium*	Erzgebirge, Central Europe, West of Dresden, Germany	S. Jessen	15
East-Ross	*A. distentifolium*	Ben Wyvis, Easter Ross NH 480 680	A. C. Jermy	30
850-L-a-M	*A. distentifolium*	Above Loch a' Mhadaidh North of Loch Fannich, 850 m Easter Ross NH 205 717	A. C. Jermy	19
CG-D	*A. distentifolium*	Corrie Garbhlach, West Cairngorms NN 88 94	H. S. McHaffie	28
GP-D-C	*A. distentifolium*	Glen Prosen, Angus, foot of slope NO 235 735	H. S. McHaffie	4
600-L-a-M	*A. distentifolium*	Above Loch a' Mhadaidh North of Loch Fannich, 600 m Easter Ross NH 199 729	A. C. Jermy	19
BA-D-A	*A. distentifolium*	Ben Alder NN 49 71 & 50 72	H. S. McHaffie	50
BA-F-B	*A. flexile*	Ben Alder NN 49 71 & 50 72	H. S. McHaffie	50
BO-D-A	*A. distentifolium*	Bridge of Orchy NN 35 43, 36 44 & 37 44	H. S. McHaffie	50
LB-D	*A. distentifolium*	Ladhar Bheinn, Knoydart NG 83 03	H.S. McHaffie	31
BO-F-A	*A. flexile*	Bridge of Orchy NN 35 43, 36 44 & 37 44	H. S. McHaffie	50
Craig-an-D-1	*A. distentifolium*	Craig an Duine North of Loch Fannich NH 20 72	A. C. Jermy	33
Craig-an-D-2	*A. distentifolium*	Craig an Duine North of Loch Fannich NH 200 729	A. C. Jermy	18
CM-F-A	*A. flexile*	Creag Meagaidh, Glen Spean NN 432 884	H. S. McHaffie	17
CM-D-E	*A. distentifolium*	Creag Meagaidh, Glen Spean NN 432 884	H. S. McHaffie	20
BE-D-A	*A. distentifolium*	Beinn Eibhinn, Glen Spean NN 452 35	H. S. McHaffie	30
BE-F-C	*A. flexile*	Beinn Eibhinn, Glen Spean NN 452 35	H. S. McHaffie	26
GE-D-A+F	*A. distentifolium/ flexile*	Glen Einich Corrie Dhondail, West Cairngorms, NN 92 97	H. S. McHaffie	33
GP-D-A	*A. distentifolium*	Glen Prosen, Angus NO 237 736	H. S. McHaffie	26
GP-F-A	*A. flexile*	Glen Prosen, Angus NO 237 736	H. S. McHaffie	20
GP-F-B	*A. flexile*	Glen Prosen, Angus NO 237 736	H. S. McHaffie	4
Pyrenees	*A. distentifolium*	Col de Puymorens, France	S. Jessen	26

6.3.3: Results

The larger sample confirmed the pilot study and showed that there were no differences between the banding patterns of *A. flexile* and *A. distentifolium*.

A. filix-femina that was included on some of the gels (Figure 6.3.1) proved to be very distinct, illustrating the difference that can occur between species.

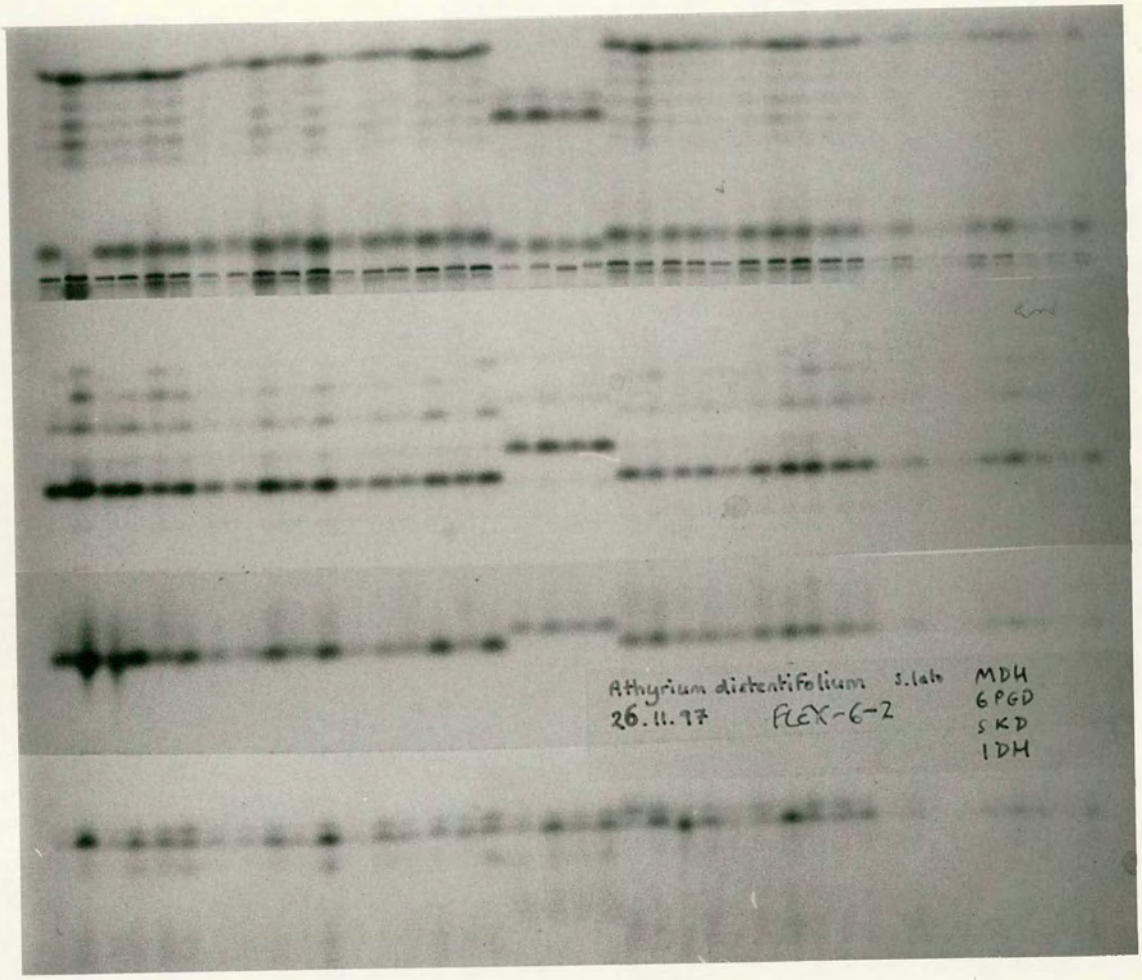
Figure 6.3.1 Malate dehydrogenase (MDH), 6-Phosphogluconate dehydrogenase (6 PGD), Shikimate dehydrogenase (SKdH) and Isocitrate dehydrogenase (IDH) staining for *Athyrium distentifolium* from North-west Scotland with four *A. filix-femina* samples in the centre of each gel.

Lane 1-3 *A. distentifolium* Fuar Tholl

Lane 4-18 *A. distentifolium* Loch a' Mhadaidh 850 m

Lane 19-22 *A. filix-femina* Loch a' Mhadaidh 850 m

Lane 23-41 *A. distentifolium* Loch a' Mhadaidh 600 m


Athyrium distentifolium s.lato
26.11.97 FLEX-6-2
MDH
6PGD
SKD
IDH

Where fronds collected from a single area gave identical patterns for all enzyme systems, and where any system showed heterozygosity, it is assumed that this represents a single clone. Two plants of *A. flexile* one metre apart at Corrie Dhondail appeared to be the same clone. At Creag Meagaidh there were three different clumps of *A. flexile* (Figure 6.3.2, upper gel, lanes 29-39), of which the largest had 11 crowns within a radius of 50 cm. Each clump was a clone. Another clump from the same locality was made up of different plants (Figure 6.3.2, lower gel, lanes 3-7).

Figure 6.3.2 Phosphoglucoisomerase (PGI) staining for *A. distentifolium* and *A. flexile* from different populations in Scotland and Switzerland scored for two loci and for three variable alleles at the lower locus. Illustrating three clones from Creag Meagaidh.

Upper gel

Lane 1-4 *A. distentifolium* Fuar Tholl

Lane 5-28 *A. distentifolium* from Ladhar Bheinn

Lane 29-32 *A. flexile* Creag Meagaidh Clump 1

Lane 33-36 *A. flexile* Creag Meagaidh Clump 2

Lane 37-39 *A. flexile* Creag Meagaidh Clump 3

Lower gel

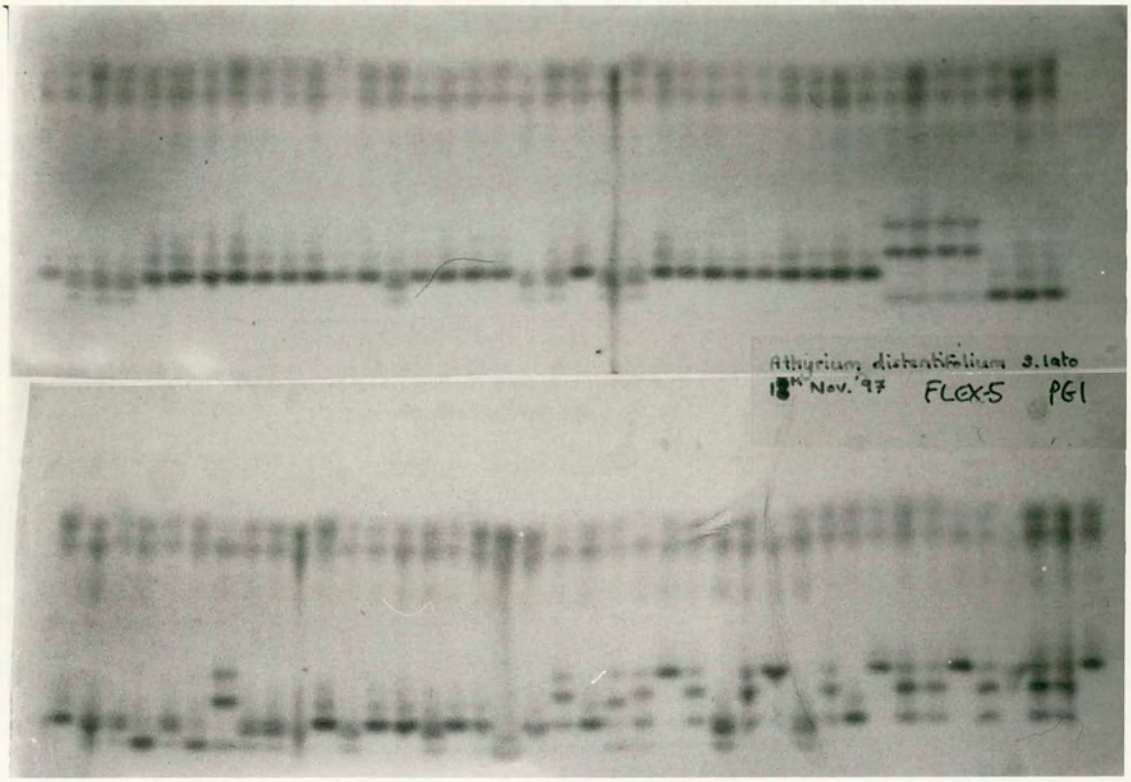
Lane 1-2 *A. distentifolium* Fuar Tholl

Lane 3-7 *A. flexile* Creag Meagaidh

Lane 8-19 *A. distentifolium* Creag Meagaidh

Lane 20-29 *A. distentifolium* Switzerland

Lane 30-40 *A. flexile* Glen Prosen


Athyrium distentifolium s.lato
18th Nov. '97 FLEX-5 PGI

A. flexile plants some distance away from other plants of either taxon might be expected to have resulted from intragametophytic selfing and be homozygous. A single plant at Ben Alder (Figure 6.3.3), and other isolated examples, like the *A. flexile* at Corrie Dhondail and a single plant at Bridge of Orchy were heterozygous for at least one enzyme system and therefore the result of outbreeding.

Figure 6.3.3 Phosphoglucoisomerase (PGI) staining for *A. distentifolium* and *A. flexile* from Ben Alder scored for two loci and for three variable alleles at the lower locus. Selected to illustrate an isolated heterozygous plant of *A. flexile* that was 100 m away from other observed *A. flexile.*

Upper gel

Lane 1-40 *A. distentifolium* Ben Alder from separate sub-populations

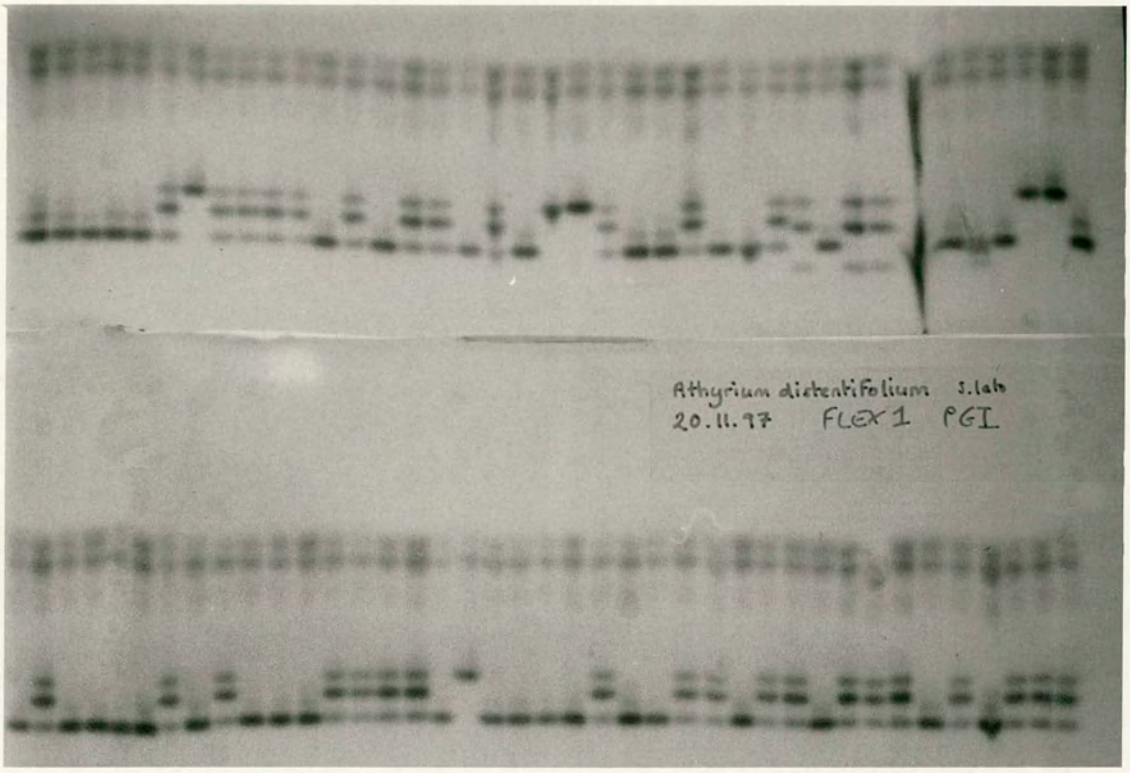
Lower gel

Lane 1-2 *A. distentifolium* Ben Alder

Lane 3-6 *A. distentifolium* Ben Alder single homozygous clone with one allele

Lane 7 *A. flexile* Ben Alder heterozygous single plant showing two alleles and a heterodimeric band

Lane 8-40 *A. flexile* Ben Alder from separate sub-populations


Athyrium distentifolium s.lab
20.11.97 FLEX 1 PGI

Nearly all the Glen Prosen population are within a radius of 12 m around the sheltering boulders (Figure 3.1). Twenty metres down the slope is a single multicrowned clump of *A. flexile*, 28 cm across, which proved to be homozygous and might have resulted from intragametophytic fertilisation (Figure 6.3.4).

Figure 6.3.4 Phosphoglucoisomerase (PGI) staining for *A. distentifolium* and *A. flexile* Alder scored for two loci and for three variable alleles at the lower locus. Selected to show a homozygous clump of *A. flexile*.

Upper gel

Lane 1-5 *A. distentifolium* Switzerland

Lane 6-15 *A. flexile* Glen Prosen monitored plants

Lane 16-25 *A. distentifolium* Glen Prosen monitored plants

Lane 25-40 *A. distentifolium* Glen Prosen

Lower gel

Lane 1-5 *A. distentifolium* Switzerland

Lane 6 *A. distentifolium* Glen Prosen heterozygous individual

Lane 7 *A. distentifolium* Glen Prosen homozygous individual

Lane 8-10 *A. flexile* Glen Prosen homozygous single clone

Lane 11-14 *A. distentifolium* Glen Prosen (foot of slope)

Lane 15-18 *A. distentifolium* Bridge of Orchy

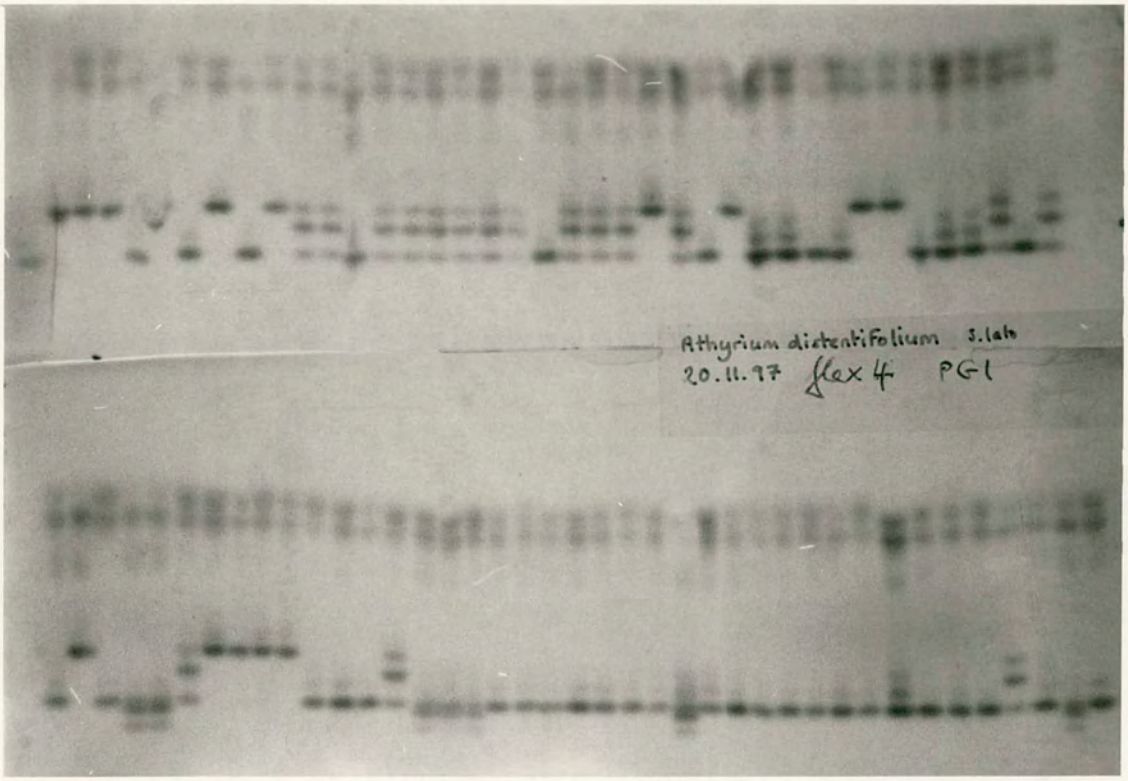
Lane 19 *A. flexile* Bridge of Orchy

Lane 20 -25 *A. distentifolium* Bridge of Orchy

Lane 26-30 *A. flexile* Bridge of Orchy

Lane 31-36 *A. distentifolium* Fuar Tholl

Lane 37-40 *A. flexile* Ben Alder


Athyrium distentifolium s.lat
20.11.97 flex 4 PG1

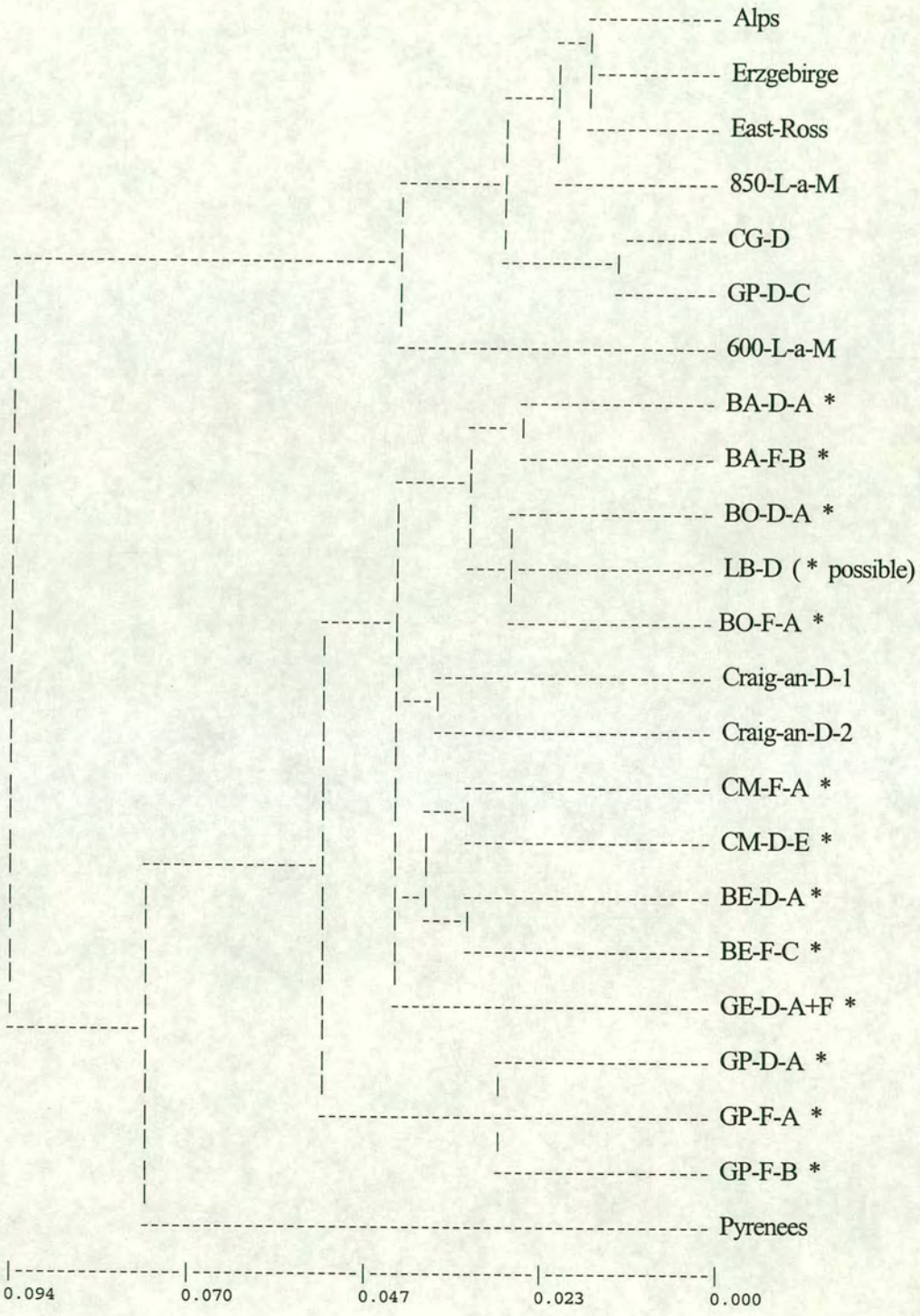
Although almost the same alleles are shared between all populations in all countries, the frequency of their occurrence is different. A cluster analysis based on presence or absence and allele frequency divided the populations into three groups (Figure 6.3.5).

The first group includes the two Central-European populations of *A. distentifolium* with four Scottish populations of *A. distentifolium*. The second group links all the populations where *A. distentifolium/flexile* occur together, with one Scottish population where *A. flexile* has not been found, and a Scottish population with a doubtful old record of *A. flexile*. The final population from the Pyrenees has one allele not shared by the other populations and has been separated in the analysis. The analysis suggests that populations of *A. distentifolium* that have the "*flexile* gene" are different from other Scottish *A. distentifolium* and different from Continental *A. distentifolium* (Figure 6.3.5).

With the exception of one separate group of only four *A. distentifolium* from Glen Prosen, the same alleles are found in both Scottish taxa from the same site. The monitored plants from Glen Prosen (Figure 6.3.4 top row lanes 6-25) show similar variation in the first ten *A. flexile* as in the succeeding ten *A. distentifolium*, although a higher proportion of *A. flexile* are homozygous in this instance. There is almost no variation between taxa within populations but there is a variation in the allele frequency between populations. This indicates that populations in separate corries have been isolated for some time and are not freely interbreeding.

The inclusion of Ladhar Bheinn among the populations that include *A. flexile* accords with a doubtful herbarium specimen. The other site from north of the Great Glen, Craig an Duine, has no records of *A. flexile*, but this indicates that there are possibly many areas which have not been thoroughly searched. The Ben Alder plants are grouped nearest to the Bridge of Orchy plants, and these sites face one another across Rannoch Moor. Although Beinn Eibhinn the nearest site to Ben Alder, it is more similar to Creag Meagaidh, and they face one another across Glen Spean.

Figure 6.3.5 Tree showing three groupings of populations with, (marked *), and without the *A. flexile* taxon. Coding as in Table 6.3.1. UPGMA phenogram



Glen Einich is an isolated site, as indeed it is geographically. Glen Prosen is also not closed linked by allele frequency with any of the other sites and is some considerable distance away from presently known sites.

6.3.4: Discussion and conclusions

The banding patterns for *A. filix-femina*, compared with *A. distentifolium*, show the differences that can be seen on an isozyme gel between different species (Figure 6.3.1). As *A. flexile* can be derived from a recessive gene or genes associated with *A. distentifolium*, these two taxa appear to be too closely interrelated to show this order of difference, at least with the enzyme systems which were used. The morphology and different growth responses of *A. flexile* indicate that there are some differences, but the distinction between *A. flexile* and *A. distentifolium* was not detected by isozyme electrophoresis.

What was detected was the variation between populations (Figure 6.3.5). The cluster analysis separated the Scottish populations of *A. distentifolium* that have *A. flexile* from the Scottish populations that apparently only have *A. distentifolium*. The *A. distentifolium*-only populations were more similar to continental populations than the Scottish ones. It appears that *A. distentifolium/flexile* populations are unique to Scotland. As a site not known to have *A. flexile* was also included in the *A. distentifolium/flexile* group, this type of *A. distentifolium* could be more widespread than the present distribution of *A. flexile* might suggest. While the populations formed two main groups in the cluster analysis, the differences between individual populations indicated by allele frequency show that every population is different from another. There is a geographical separation between the various locations and this probably dates from the end of the most recent phase of glaciation at least ten thousand years ago.

Most populations of ferns analysed by Soltis and Soltis (1989) were outcrossing and agreed with the Hardy-Weinberg predictions. They found that these outcrossing

species with a free gene flow have similar genetic variation across a wide part of their range. Each population of *A. distentifolium/flexile* that was studied was freely outcrossing, but the differences between these populations show that the outcrossing was generally confined within the corrie.

The isolation between populations might assist in explaining the present-day distribution of populations with *A. flexile* that appears to be confined to parts of the Highlands of Scotland. A single mutation might have spread, or the same mutation might have arisen in different populations. The geographical proximity suggests a common origin, possibly associated with the end of glaciation when the habitat would have been more widespread. It is possible that the A. *distentifolium/flexile* populations might have survived glaciation on unglaciated areas and the "pure" *A. distentifolium* populations could have colonised postglacially. The postglacial fragmentation of *A. distentifolium* habitats appears to have divided Scottish populations of *A. distentifolium* into those that have the "*flexile* gene" and those that do not. While long-distance spore dispersal remains possible, the chances of *A. flexile* spreading appear limited as new plants currently apparently arise very infrequently.

Individual plants have already been found to possibly be decades old (4.8). This was confirmed by the Creag Meagaidh clones (Figure 6.3.2) which indicated the slow spread of one original plant. With so few single-crowned plants within a population there might not have been any new recruitment for many decades. Plants that make up the present colonies appear to be long-established populations. A single plant of *A. flexile* near Bridge of Orchy, 200 metres from the nearest *A. flexile* and several metres from the nearest *A. distentifolium*, was found to have formed by outcrossing in a similar way to the isolated plant at Ben Alder (Figure 6.3.3). This revealed that this sporophyte was not formed by a single self-fertilised gametophyte, like the example from Glen Prosen (Figure 6.3.4), but the product of fertilisation between two gametophytes with the recessive "*flexile* gene". Although a considerable time might pass before two gametophytes grew close enough to cross-fertilise, the spore

rain must have been sufficient to provide two gametophytes in the right place at the right time.

Some of the records for *A. flexile* are for single plants (Table 2.1) and they have been recorded from areas that currently are not known to have large populations of *A. flexile.* As the populations of *A. distentifolium* with the "*flexile* gene" are probably more widespread than is realised, single plants might have occurred through gametophytes with the "*flexile* gene" coinciding, but some areas, like Ben Alder, must have offered especially favourable conditions at the time of colonisation for such a high proportion of *A. flexile* to be present. The differences in allele frequencies that have evolved between the populations demonstrate that they must have reproduced more in the past, after they were isolated. Similar though the *A. flexile* morphology is between sites, the most morphologically varied population is found at Bridge of Orchy and many generations might have succeeded one another to produce the plants that are now present.

Only three populations of more than 100 plants of *A. flexile* are known. The existence of single plants could indicate that this taxon might have been more widespread and raises the question whether its range has been reduced or extended. Climatic variation over a long time scale might provide a more suitable habitat for one or other taxon. Large clumps of both taxa suggest that the present sporophytes are very old and the last major episode of colonisation might have been connected to disturbance associated with the Little Ice-age more than 300 years ago. The plants that are observed now could be the result of colonisation under different circumstances that no longer prevail, for the scree environment is currently relatively stable with little opportunity for colonisation.

6.4 Conclusions

Selfing experiments showed that intragametophytic selfing is possible, although there appears to be some genetic load. The isozyme analysis showed many examples of homozygous and heterozygous plants in the field, confirming that both outcrossing and self-crossing do occur.

Pairs of gametophytes of either *A. distentifolium* or *A. flexile* used for the crossing experiments (Table 6.2.1) were mostly observed to both be hermaphrodite, and yet successful fertilisation often did not occur. While laboratory experiments can suggest mechanisms that might operate in wild conditions, they are necessarily artificial. Nevertheless, some generalisations can be made from observations that would be difficult to make in the field. Many factors were observed to influence successful fertilisation. The pH of the growth medium affected the gender, as did the presence or absence of specific minerals. The temperature had an influence on gender, as did the presence of antheridiogen and might the density of the culture. The genetic load of the various source plants would also influence the potential ratio of outcrossed individuals. The infrequent records of single plants of *A. flexile* in scattered localities imply that the *flexile* combination might be an unusual event. Isozyme evidence suggested that *A. distentifolium/flexile* populations could be more widely distributed than previously supposed. The substantial numbers of the *flexile* taxon at Ben Alder, Beinn Eibhinn and Bridge of Orchy suggest than in these particular locations strong selective pressure appears to have taken effect in response to local conditions.

Fuar Tholl in Strathcarron in the North-west has small plants of *A. distentifolium* and would appear to offer a suitable site for *A. flexile*. This location was included in the isozyme analysis but the data set was inadvertently omitted from the cluster analysis. The allele frequency will give an indication of whether this could be an *A. distentifolium/flexile* site. P. Hainsworth (pers. comm, 1997) has searched over many years without locating *A. flexile*. This might be an example of a suitable habitat that does not have the "*flexile* gene" and thus the distribution has been restricted.

CHAPTER SEVEN Discussion and Conclusions

7.1: Summary

During this study the habitat shared by *A. distentifolium* and *A. flexile* was characterised. Both taxa grow together on an acidic, low-nutrient substrate, with a pH range of 3.5-4.5 (3.2.5). An extended snow cover provides a specialised environment that only a limited range of plants can tolerate and this reduces the competition (3.2.4). The need for frost protection by snow was demonstrated by the death of plants in freezing experiments (3.4.3) and by the frosted fronds at Glen Prosen that started to grow in an early spring (3.5). The high altitude of these sites ensures low temperature for most of the summer, with a mean ground temperature of around 10 °C ((3.2.3), although these arctic-alpine ferns can make use of higher temperatures for rapid growth.

A. distentifolium and *A. flexile* occur in two main types of plant communities. Firstly, the *Cryptogramma crispa - Athyrium distentifolium* snowbed community (Rodwell, 1992) that is typical of extended snow cover and the vegetation is generally low. Secondly, both taxa can also occur in a tall-herb community. This can be represented by dense fern meadows and almost pure stands of *A. distentifolium*, but in areas like the Bridge of Orchy site, the vegetation is neither too tall not too dense and *A. flexile* is able to compete (3.3). The lack of competition from *A. distentifolium* is essential for *A. flexile* to succeed.

Morphometric analysis on fronds of both taxa showed that *A. flexile* is morphologically distinct. In a principal components analysis a distinction was noted in the range of characters of *A. distentifolium* from Scotland compared with *A. distentifolium* from Europe and Scandinavia (4.2). While many of the Scottish *A. distentifolium* fronds were indistinguishable from those of other countries on the first two components, others, particularly from the *A. flexile* sites, were nearer to

A. flexile and made a link between the non-Scottish *A. distentifolium* and *A. flexile*. In comparisons of frond height, the Scottish *A. distentifolium* were frequently smaller than non-Scottish (4.3), adding to the suggestion that some Scottish *A. distentifolium* is different, or has grown under less favourable conditions. A higher number of cells in the annulus was discovered as a useful taxonomic feature for the confirmation of *A. flexile* (4.4). The *A. distentifolium* populations where *A. flexile* has not been found, have a lower number of cells than the populations where both taxa were found together. This indicated the sharing of joint characters in populations where both taxa were present.

Growth experiments demonstrated differences between *A. distentifolium* and *A. flexile.* Most of the experiments investigated germination and gametophyte growth using a gel medium of pH 3.8, that approximated to the acidic substrate in the field. Some *A. flexile* gametophytes grew considerably larger than many *A. distentifolium* gametophytes (5.3), suggesting that there might be a competitive advantage in a low nutrient environment even at the gametophyte stage. Gametophytes grown on media with specific nutrients absent also showed that *A. flexile* gametophytes grew larger than *A. distentifolium* (5.3) and illustrated the capacity to grow in impoverished conditions. Sporophytes of both taxa showed the different responses to low nutrients as *A. distentifolium* required a high level of nutrient to grow vigorously and become fertile while *A. flexile* sporophytes were precociously fertile (5.4). This verifies that *A. flexile* is particularly well adapted to the low-nutrient habitat that restricts the growth of *A. distentifolium.*

Gametophytes of *A. flexile* grew particularly well in a nutrient medium without phosphorus and potassium (5.3). The mycorrhiza study showed that sporophytes can have a high level of colonisation by mycorrhiza (4.9). One of the benefits of a mycorrhizal association, is the increased uptake of phosphorus by the symbiont. The fertility of *A. flexile* sporophytes (5.5) indicated that many plants were receiving adequate nutrients in the wild. But *A. distentifolium,* also with high levels of colonisation by mycorrhiza, had a trend towards a negative correlation with fertility

(4.9), implying that this habitat, even with the mycorrhizal assistance, was not providing sufficient nutrients for this taxon. *A. flexile* gametophytes that have established in a nutrient-deficient environment should have less competition from *A. distentifolium* gametophytes, and any sporophytes would have a greater chance of success. If the gametophytes can establish in a site with low phosphorus, this need not be a limiting factor for the eventual sporophyte as mycorrhizal colonisation was frequent.

In morphology, growth habit and edaphic preferences, *A. flexile* can be seen to be distinct from *A. distentifolium*. When spores from certain *A. distentifolium* plants were sown, it was found that the resulting progeny could be either *A. distentifolium* or *A. flexile* (5.4). This appears to be the result of the expression in *A. flexile* of a homozygous recessive gene, or set of closely linked genes, that are only found in polymorphic populations of *A. distentifolium* (5.6). Isozyme analysis (6.3) found more variation between separate populations, than between the two taxa within each population. Scottish populations of *A. distentifolium* were found to divide into those that included *A. flexile* and those where this taxon had not been found. Plants from the non-*flexile* sites were more similar to those from two European locations. This supports the suggestion that two types of *A. distentifolium* are present in Scotland. The polymorphic populations may have *A. flexile* present but the other "pure" *A. distentifolium* does not. If *A. flexile* is the product of a genetic combination from a freely interbreeding population, its species status must be questioned.

7.2: Review of the status of *A. flexile*

One of the most frequently used definitions of a species is the biological one. Mayr (1969) defined species as "groups of interbreeding natural populations that are reproductively isolated from other groups". By this definition, *A. flexile* and *A. distentifolium* are able to interbreed and should be classified as the same species. Some species that have been reproductively isolated, usually geographically, like *Hyacinthoides non-scripta* and *H. hispanica* (Miller) Rothm, can interbreed when they are grown together and produce intermediate fertile hybrids. *A. flexile* and

A. distentifolium are always found together and are not geographically separated. It is possible that *A. flexile* might have been separated during glaciation and they have come together again, although there is no evidence for this.

One possible explanation for these data is to regard *A. distentifolium* and *A. flexile* as separate species that can freely hybridise. The *A. distentifolium* plants that can produce either taxon could be fully fertile hybrids. Hybrids can usually be distinguished from the parent species by intermediate features. This is not possible in these populations where individuals can normally be clearly assigned to one taxon or the other, although intermediate specimens have been grown from spores (5.4). The number of cells in the annulus might indicate which *A. distentifolium* plants were hybrids, but there is no consistent pattern distinguishing the plants that are known to produce both taxa, compared with those that breed true. During hybridisation, *A. flexile* could behave as a recessive to *A. distentifolium* and the same genetic mechanism would allow back-crossing in the second generation. If they do hybridise, the essential characters which distinguish *A. flexile* must be strongly linked for this taxon would long since have become amalgamated with *A. distentifolium* and only a wide range of intermediate morphologies produced, like hybrid *Hyacinthoides*. This has not happened as field specimens are generally clearly distinguishable as one type or the other.

Although derived from *A. distentifolium*, Page (1997) has suggested which *A. flexile* could be the potential origin of a new species and of particular interest as an example of evolution in action. A physical separation between the taxa would be necessary to ensure complete separation as they are currently able to freely interbreed. Although *A. flexile* performs better in low-nutrient environments, it always occurs with *A. distentifolium* and it is difficult to envisage circumstances that would isolate *A. flexile* without *A. distentifolium*. *A. flexile* would have to demonstrate the capacity to survive in circumstances that *A. distentifolium* could not tolerate.

Haufler (1996) referred to the morphological species concept where individuals could be classified through observable discontinuities in form. This is possible with *A. flexile* and *A. distentifolium,* for despite the appearance under cultivation of a greater variety of morphologies than are seen in the field, most sporophytes can be easily assigned to one or the other taxon (4.2). The original classification and separation as a species was based on morphology, but in the light of the findings detailed in this thesis, morphology alone is not an adequate basis for division. Cultivation experiments (5.4) have suggested a mechanism for the production of *A. flexile* from *A. distentifolium* plants, and it is proposed that the distinctive morphology is the product of a recessive genetic combination to give what is usually classified as a variety. This accords with the alternative varietal name *A. distentifolium* var. *flexile* (Newman) Jermy (Jermy *et al*, 1978), and supports the view of James Backhouse who originally found it, and considered it was a variety (Moore, 1859). A distinction does, however need to be made between the *A. distentifolium* that occurs in the polymorphic *A. distentifolium/flexile* populations and other "pure" *A. distentifolium* that does not apparently have the range of genetic variation that includes *A. d.* var. *flexile.* There are thus three varieties of *A. distentifolium* present in Scotland.

7.3: An endemic ecotype

Isozyme analysis suggested (6.3) that the "*flexile* gene" is only found in Scotland. The distinctive morphology has not been reported from elsewhere, despite an awareness of its existence by competent botanists. Many cultivated fern varieties look very different from the typical ferns but may be the result of selective breeding and many can only be propagated vegetatively or require careful nurturing to survive at all. However, *A. distentifolium* var. *flexile* not only looks dissimilar, it also has a different growth strategy and ecology to the other two varieties of *A. distentifolium.*

It is feasible that the *A. distentifolium* in the polymorphic populations may be better adapted than the "pure" *A. distentifolium* to growth in poor conditions. However monitored plants of *A. distentifolium* at Glen Prosen that were found to be able to

produce both types, were extremely vigorous. This suggested that there is no upper growth limit. *A. d.* var. *flexile*, on the other hand, always has shorter fronds, even at Bridge of Orchy in the site with the best nutrition. Growth experiments with different nutrient levels (5.4) showed that *A. d.* var. *flexile* used additional nutrient to be more fertile and produce twice as many fronds, but the overall height was little different compared with the growth at half the nutrient level. This implies *A. d.* var. *flexile* has a dwarfing component in the genetic "package". Among the *A. distentifolium* that was cultivated from polymorphic and non-polymorphic populations, no outstanding differences were observed. Of all the *A. distentifolium* in cultivation, fertility was induced only with the application of significant amounts of nutrient, far in excess of the amounts that *A. d.* var. *flexile* required.

A. d. var. *flexile* is found in areas that experience extreme weather conditions, even through the summer months. Its small size and early maturity help it to thrive in a short, cold season. The evidence suggests that *A. d.* var. *flexile* can only grow where the nutrient levels are low enough to reduce the height and fertility of *A. distentifolium* to a level with which *A. d.* var. *flexile* can compete. As the distribution of snow-bed communities is restricted, and *A. d.* var. *flexile* is only known in substantial numbers from three sites, there may not be many sites that satisfy the range of conditions that it requires, although there might be other populations that have not been located. The height of the other vegetation, and *A. distentifolium* in particular, will have a strong bearing on the potential sites for *A. d.* var. *flexile*.

Although these taxa grow in close proximity, there is an ecological separation between them. *A. d.* var. *flexile* is less likely to occur in high-nutrient areas, where it is the weaker competitor. Tall, vigorous *A. distentifolium* would undoubtedly shade *A. d.* var. *flexile* excessively and lead to its eventual elimination from a population. These taxa are only found together where the growth of *A. distentifolium* is restricted by the very conditions that allow *A. d.* var. *flexile* to reach its full height and be fertile.

It is suggested that *A. d.* var. *flexile* is a specially adapted ecotype. While there are Scottish locations with dense stands of lush *A. distentifolium*, there are also sites where the Scottish combination of oceanic and continental climate gives erratic seasons with a different environment from the more predictable continental pattern. *A. distentifolium* at its best is a tall-growing competitor, typical of undisturbed sites. Grime and Hunt (1975) compared the rates of growth of a wide range of plants and outlined different strategies for the competitive herbaceous plants. Perhaps most applicable to the taller growing *A. distentifolium* is the adoption of a ". . tall stature, extensive lateral spread and the tendency to accumulate leaf litter, all characteristics that facilitate the exclusive occupation of productive, undisturbed habitats". *A. d.* var. *flexile,* in contrast, is a stress-tolerator, able to grow and be fertile even with low soil nutrients. Grime (1979) observed that the same plant in one site may not be so competitive in another and this is seen in the smaller, less fertile *A. distentifolium* which occurs in the *A. distentifolium/flexile* populations. The small stature of *A. d.* var. *flexile* is advantageous in so far as *A. distentifolium* cannot achieve a reproductive size with low nutrients. Low growth also assists in low wind resistance and the bend at the base of the stipe that gave *A. d.* var. *flexile* its name could be a useful adaptation.

The tolerance of greater extremes is continued in the apparent tendency of *A. d.* var. *flexile* to occur in the areas of relatively longer snow-lie (3.2.4). This does not approach the length of snow-lie of Norwegian plants that may not be uncovered until August (Odland, 1991), but those particular populations grow in more basic conditions. With an excessively short season, plants growing in a low-nutrient soil would not make adequate growth either for reproduction or to replenish reserves before the following winter. While Scottish snow cover can be extended in some seasons, the period is less predictable. The small size, rapid growth, and potentially earlier maturity (5.5), assist the *flexile* ecotype in these extreme communities. The darker green colour of *A. d.* var. *flexile* with more prostrate fronds also implies an adaptation to growth in more shaded conditions, possibly from

greater cloud cover and among sheltering rocks. The upright habit of *A. distentifolium* forms a marked contrast and is more suited to growth in dense stands or in montane scrub, the habitat in which many population occur in Scandinavia.

Flowering plants also have different ecotypes that are morphologically different but are classified as the same species. Crawford and Smith (1997) compared the growth strategies of *Saxifraga oppositifolia* L. plants in Greenland growing on beach ridges with those that grew on the low shore. The beach-ridge plants were more robust and had a growth form that was almost upright. The flowers had overlapping petals and although the season was short, they produced seed. The ridge was snow-free before the lower areas, but there was the danger of drought. These plants had a tap root and a low rate of respiration. The low-shore plants had no tap root, a prostrate growth habit, the petals did not overlap and the flowers produced pollen that could fertilise the beach-ridge plants but often did not have a long enough season to produce seed. Their respiration rate was very high as they had adequate water, but a short growing season. Although both growth forms, with intermediates, were present on both sites, the robust form was predominant on the ridges and the prostrate form in the hollows. Crawford *et al.* (1995) suggested that the ridge plants are normally outcrossing and that a range of progeny are produced. Seed is deposited over the whole habitat and the most appropriate type of plant for that particular habitat would grow more vigorously.

This is similar to *A. d.* var. *flexile* and *A. distentifolium* but the habitats are not so clearly differentiated. Although Glen Prosen is a very small site it perhaps gives the best illustration. In the centre of the site there is tall, well-grown *A. distentifolium* and the smaller *A. d.* var. *flexile* would not be able to compete. The spores from several of these plants can produce either *A. d.* var. *flexile* or *A. distentifolium* (5.4). Any spores landing in the immediate vicinity of the large clumps are unlikely to germinate if the decaying fronds emit a leachate that inhibits germination (5.3). The fronds are also very dense and small plants of either taxon would be excessively shaded. Only on the

edge of the site were smaller plants of both taxa able to establish, of which only one *A. distentifolium* plant has been observed to be fertile. Half of the *A. d.* var. *flexile* plants were fertile, an unusually low number for *A. d.* var. *flexile*, implying the edge does not offer an optimum growing environment. In these circumstances both taxa experience the same conditions, but only *A. d.* var. *flexile* is able to reproduce. This might also increase the proportion of *A. d.* var. *flexile* plants present as they will produce more spores.

At other sites, both taxa grow intermixed. The height of *A. distentifolium* is variable, especially at Bridge of Orchy, and this taxon might occupy more enriched pockets. Like the *S. oppositifolia,* when spores of both types are produced they would grow better if they establish in a site that more nearly meets their requirements. The range of intermediates seen in *S. oppositifolia* is not apparently produced in such abundance as are found in cultivated *A. distentifolium* from polymorphic populations. It is possible that many intermediate plants do occur, but they may not survive. The genetic variation is nevertheless available should any plants have a particularly advantageous growth form for a particular habitat.

During many growth experiments, Turesson (1922) found more genetic diversity in a wide range of flowering plants than had been observed in the wild populations. Most common plants exhibit a range of adaptations. While local ecotypes are specially adapted to local conditions, Turesson (1922) proposed that there is enough overlap between adjacent habitats to ensure intercrossing between the different growth forms. This prevents breeding barriers from forming as there is no isolation of one particular type. Outcrossing maintains genetic diversity and the ability to respond to environmental change. Turesson (1922) linked extreme habitats to the occurrence of very specific modifications. Some adaptations may only be induced by the degree of exposure, and the growth habit is no longer seen in cultivated specimens. Other adaptations, or a group of genetically determined attributes, are collectively advantageous. This is applicable to *A. d.* var. *flexile*, which is not only morphologically different from *A. distentifolium*, but is able to grow successfully

conditions that restrict *A. distentifolium*. The range of variation observed in cultivated specimens has not been seen in the field. While a certain amount of natural variation has been observed, the observed range must be limited by the specific environment. Turesson (1922) called this ". . the reaction-types of the ecotypes arising through the modification influence of the combination of extreme habitat factors given in nature". The capacity to outcross with *A. distentifolium* helps *A. d.* var. *flexile* to maintain genetic variation, at least within the corrie as there is apparently little genetic exchange between adjacent corries (6.3).

The *flexile* ecotype occupies a specific habitat that is the result of a combination of the Scottish climate and acidic rock type. The populations of polymorphic *A. distentifolium* that gave rise to the *flexile* taxon appear to be endemic to Scotland. Morphologically this *A. distentifolium* looks very similar to fronds from non-*flexile* populations. Given adequate nutrient, *A. distentifolium* from these mixed populations can grow as vigorously as any other *A. distentifolium*, but because some sites are so nutrient deficient this has allowed the *flexile* ecotype to establish. Similar habitats may well exist elsewhere in the world, but if the "*flexile* gene" is not present, then only small *A. distentifolium* will be found.

7.4: Future studies

The precise distribution of the *A. distentifolium/flexile* populations is still not known in detail. It would be possible to sample populations to assess the presence of the "*flexile* gene", by growing spores to determine the presence of both types of sporophyte, by counting the cells in the annulus, or through isozyme analysis. More field work would extend the known distribution of the *flexile* ecotype, particularly an intensive search in areas that appear suitable but have no former records. Further samples of *A. distentifolium* from Europe and Scandinavia would provide more evidence for the post-glacial sequence of colonisation. In addition to the appearance of a specialised endemic variety in Scotland, it is interesting to compare the genetic variation of populations of a widespread species that currently have little opportunity to interbreed.

Growth experiments suggested that the gametophytes of both taxa respond to different nutrients. Spores from *A. distentifolium* plants known to produce both varieties could be sown on substrates with specific nutrients. It is possible the ratio of sporophytes obtained would vary according to which is most appropriate for the taxon. No attempt has been made to compare the ratio of sporophytes obtained by mixed mating at different temperatures and this could be another interesting line of enquiry.

A. distentifolium is a difficult plant to grow successfully in cultivation and the nutrient cycle of plants growing in a scree with very little soil would be of considerable interest. The adaptation to a low-nutrient habitat offers scope for further work. Many of the plants sampled had high levels of mycorrhizal colonisation (4.9) and this is an important factor that has been little studied in the field, both in ferns generally or in high altitude plants.

7.5: Conclusions and recommendations

As there are many polymorphic populations of *A. distentifolium* with the potential capacity to produce *A. d.* var. *flexile*, the "*flexile* gene" should exist for some time to come and this taxon is not in any immediate danger. At Glen Prosen, the original site, the plants of *A. d.* var. *flexile* were apparently collected out by 1886 (Boswell *et al*, 1888). The present population demonstrates the ability to recolonise during the last 100 years. Natural climatic variation can highly modify the vegetation, but possibly the greatest influence has been anthropogenic through the management of grazing that appears to have restricted the range of these arctic-alpine communities either to ledges or less-accessible scree.

Odland (1995) referred to fern meadows in Norway but there are few equivalent Scottish examples. Without such intensive grazing, these habitats that have become restricted to the cliff ledges could still be available. A survey of fifty plants each of

A. d. var. *flexile* and *A. distentifolium* at Ben Alder (3.4.1) found that 20 to 30% respectively of the clumps had been grazed in amounts varying from 5 to 90% of the foliage. Grazing was very variable depending on the terrain. Accessible plants among the smaller rocks were often grazed; those among large unstable boulders were not. This represents a significant impact over a period of time and suggests that grazing has restricted the present distribution of *A. d.* var. *flexile* and *A. distentifolium*. It is very significant that the three largest surviving populations are in areas of blocky scree.

As they have survived at the present grazing levels they are unlikely to be affected in the near future. There is no evidence that the present sites for *A. d.* var. *flexile* are dangerously threatened. Fortunately, these populations are located far from any recreational areas of development and the nearest habitations. Because the *flexile* taxon is part of the polymorphic *A. distentifolium* populations it is not necessary to apply specific conservation measures and the reintroductions suggested in the Biodiversity Action Plan would not be appropriate. This removes any need for the conservation of individual plants, or individual sites. Of greater importance is the maintenance of the whole habitat including monitoring of grazing in these sensitive high altitude sites.

With speculation about climate change it is difficult to predict how individual species will respond or which characteristics will be advantageous. With a range of genetic variation there is a higher chance that species will be able to adapt. If snow cover becomes too uncertain, the high altitude environment will remain unsuitable for species that cannot tolerate long snow-lie when it does occur, but *A. d.* var. *flexile* might prove to be able to contend with fluctuating conditions better than *A. distentifolium*. *A. d.* var. *flexile* is already adapted to growing in an extreme environment where it can make the best use of the resources available. Its smaller size and capacity to flush new fronds throughout the season, means that it would be less badly affected by late frosting and could still produce fertile fronds. The

potential for rapid production of new sporophytes compared with *A. distentifolium* could lead to increased colonisation in the event of whole populations being lost.

Several conclusions can be reached:

- *A. d.* var. *flexile* appears to be a homozygous recessive derived from polymorphic populations of *A. distentifolium*
- These polymorphic populations are endemic and are only found in the Scottish Highlands.
- Only some Scottish populations are polymorphic, others have more affinity with continental *A. distentifolium*.
- *A. d.* var. *flexile* is only successful in low-nutrient localities with reduced competition from *A. distentifolium*.
- In these circumstances *A. d.* var. *flexile* forms a recognisable ecotype with a different growth strategy compared with *A. distentifolium*.
- The polymorphic populations contained substantial long-lived clones, that can reasonably be expected to survive into the foreseeable future.
- The polymorphic *A. distentifolium* populations are probably restricted by grazing, might be affected by pollutants and are potentially affected by global warming, but are otherwise under no local or specific threat that can be ameliorated.

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Appendix Ia

Table I Source of fronds in morphometric analysis (4.2)

A. flexile

1 1995 Ben Alder 1
2 1995 Ben Alder 3
3 1995 Ben Alder 5
4 1995 Ben Alder 7
5 1995 Ben Alder 8
6 1995 Beinn a' Chreachain A
7 1995 Bridge of Orchy 2
8 1995 Creag Meagaidh 3a
9 1995 Glen Prosen 0
10 1995 Glen Prosen 2
11 1995 Glen Prosen 9
12 1995 Creag Meagaidh 3b
13 1995 Creag Meagaidh 1
14 1995 Creag Meagaidh 2
15 1995 Beinn a' Chreachain 1
16 1995 Beinn a' Chreachain 5
17 1995 Beinn a' Chreachain 2
18 1995 Beinn a' Chreachain 3
19 1995 Beinn a' Chreachain 4
20 1995 Beinn Achaladair 1
21 1995 Glen Prosen 1
22 1995 Glen Prosen 5
23 1995 Glen Prosen 8
24 1995 Ben Alder 6
25 1995 Ben Alder 4
26 1995 Bridge of Orchy 5
27 1995 Bridge of Orchy 7
28 1995 Bridge of Orchy 6
29 1995 Bridge of Orchy 3
30 1995 Bridge of Orchy 4
31 1995 Bridge of Orchy 9
32 1995 Bridge of Orchy 1
33 1995 Bridge of Orchy 10
34 1995 Bridge of Orchy 8
35 1995 Ben Alder 2
36 1995 Meall Buidhe1
37 1995 Meall Buidhe 2
38 1995 Meall Buidhe 3
39 1995 Glen Prosen 4
40 1995 Meall Buidhe 4
41 1995 Bridge of Orchy 0
42 1996 Bridge of Orchy 6
43 1996 Bridge of Orchy 5
44 1996 Bridge of Orchy 4
45 1996 Bridge of Orchy 3
46 1996 Bridge of Orchy 1
47 1996 Bridge of Orchy 2
48 1996 Bridge of Orchy 7
49 1996 Bridge of Orchy 8
50 1996 Bridge of Orchy 9
51 1996 Bridge of Orchy 10
52 1996 Glen Prosen 10
53 1996 Glen Prosen 7
54 1996 Glen Prosen 8
55 1996 Glen Prosen 6
56 1996 Glen Prosen 5
57 1996 Glen Prosen 4
58 1996 Glen Prosen 3
59 1996 Glen Prosen 2
60 1996 Beinn Eibhinn 3
61 1996 Beinn Eibhinn 6
62 1996 Beinn Eibhinn 4
63 1996 Beinn Eibhinn 7
64 1996 Beinn Eibhinn 5
65 1996 Beinn Eibhinn 2
66 1996 Ben Alder H
67 1996 Ben Alder A
68 1996 Glen Prosen 9
69 1996 Glen Prosen 1
70 1996 Ben Alder D
71 1996 Ben Alder C
72 1996 Ben Alder E
73 1996 Ben Alder B
74 1996 Ben Alder K
75 1996 Bridge of Orchy y
76 1852 Herb Glen Prosen 1
77 1852 Herb Glen Prosen 2
78 1852 Herb Glen Prosen 3
79 1852 Herb Glen Prosen 4
80 1995 Creag Meagaidh 1
81 1973 Herb Ben Alder 1
82 1946 Herb Ben Alder 2
83 1867 Herb Ben Alder 3
84 1957 Herb Beinn Eibhinn1
85 1855 Herb Glen Prosen 5

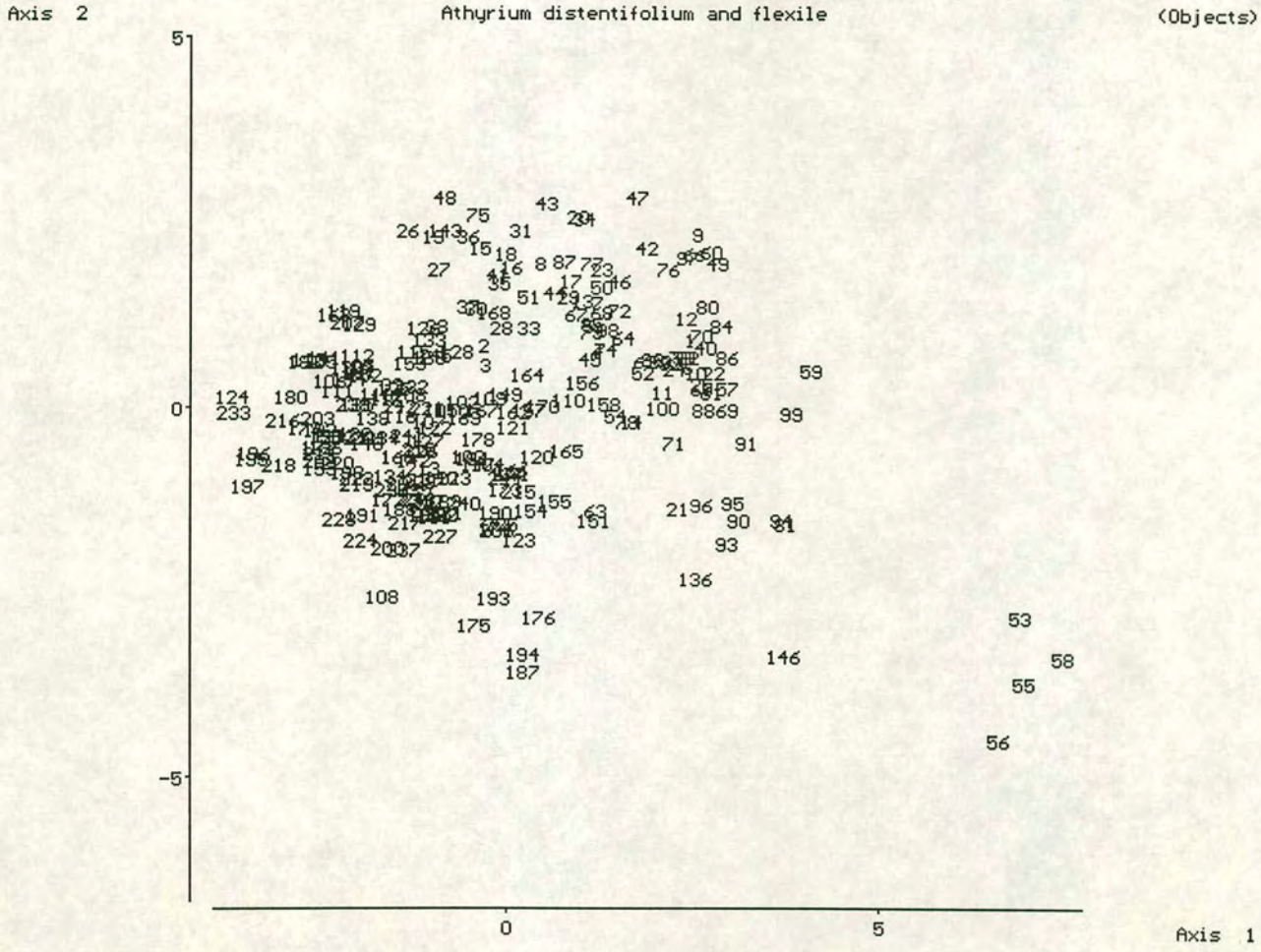
86 1867 Herb Ben Alder 4
87 1852 Herb Glen Prosen 6
88 1852 Herb Glen Prosen 7
89 1852 Herb Glen Prosen 8
90 1855 Herb Glen Prosen 9
91 1855 Herb Glen Prosen 10
92 1867 Ben Alder 5
93 1973 Ben Alder 6
94 1946 Ben Alder 7
95 1946 Ben Alder 8
96 1867 Ben Alder 9
97 1973 Ben Alder 10
98 1867 Ben Alder 11
99 1867 Ben Alder 12
100 1943 Meall Buidhe 1

A. distentifolium
101 1995 Ben Alder 6
102 1995 Ben Alder 4
103 1995 Ben Alder 7
104 1995 Ben Achaladair 1
105 1995 Bridge of Orchy 0
106 1995 Ben Alder 3
107 1995 Ben Alder 5
108 1995 Ben Alder 2
109 1995 Ben Alder 1
110 1995 Ben Alder 8
111 1995 Bridge of Orchy 3
112 1995 Bridge of Orchy 10
113 1995 Bridge of Orchy 9
114 1995 Bridge of Orchy 5
115 1995 Bridge of Orchy 4
116 1995 Bridge of Orchy 6
117 1995 Bridge of Orchy 8
118 1995 Bridge of Orchy 1
119 1995 Bridge of Orchy 2
120 1995 Bridge of Orchy 7
121 1995 Ben Lawers 2
122 1995 Meall Buidhe 1
123 1995 Meall Buidhe 2
124 1995 Meall Buidhe 3
125 1995 Meall Buidhe 4
126 1995 Meall Buidhe 5
127 1995 Meall Buidhe 6
128 1995 Meall Buidhe 7
129 1995 Meall Buidhe 8
130 1995 Meall Buidhe 9
131 1995 Creag Meagaidh 1
132 1995 Creag Meagaidh 2
133 1995 Meall Buidhe 10
134 1995 Meall Buidhe 11
135 1995 Meall Buidhe 12
136 1996 Bridge of Orchy 6
137 1996 Bridge of Orchy 7
138 1996 Bridge of Orchy 8
139 1996 Bridge of Orchy 10
140 1996 Bridge of Orchy 1
141 1996 Bridge of Orchy 4
142 1996 Bridge of Orchy 3
143 1996 Bridge of Orchy 5
144 1996 Bridge of Orchy 2
145 1996 Bridge of Orchy 9
146 1995 Glen Prosen 9
147 1995 Glen Prosen 6
148 1996 Beinn Eibhinn 2
149 1996 Beinn Eibhinn 9
150 1996 Beinn Eibhinn 1
151 1996 Beinn Eibhinn 4
152 1996 Beinn Eibhinn 5
153 1996 Beinn Eibhinn 8
154 1996 Beinn Eibhinn 6
155 1996 Beinn Eibhinn 7
156 1996 Beinn Eibhinn 3
157 1996 Beinn Eibhinn I3
158 1996 Beinn Eibhinn I1
159 1996 Ben Alder C
160 1996 Glen Doll A
161 1996 Glen Doll B
162 1996 Ben Alder A
163 1996 Ben Alder B
164 1996 Ben Alder J
165 1996 Ben Alder I
166 1996 Ben Alder H
167 1996 Ben Alder F
168 1996 Ben Alder E
169 1869 Herb Clova 2
170 1862 Herb Ben Lawers 3
171 1866 Herb Glockmore Glen 4
172 1974 Herb Creag Meagaidh
173 1938 Herb Aonach Beag 5
174 1946 Herb Ben Alder 6
175 1977 Herb Wester Ross 7

176 1956 Herb Ross-shire 8
177 1852 Herb Corrie Fee 9
178 1863 Herb Glen Doll 10
179 1867 Herb Ben Alder 11
180 1869 Herb Clova 12
181 1962 Herb Corrie Fee 13
182 1871 Herb Glen Doll 14
183 1940 Am Binnein Ben More 15
184 1946 Herb Strath Oykell 16
185 1853 Herb Glen Shee 17
186 1896 Herb Sweden Jampland 4
187 1923 Herb Sweden 1
188 none Sweden Lake Torneträsh 2
189 1960 Sweden 3
190 1888 Lapland 1
191 none Austria Senneralpe 1
192 1971 Norway 3
193 1962 Norway N. of Vinge 1
194 1962 Norway N. of Vinge 2
195 1828 Herb Switzerland Kunze 5
196 1863 Herb Lapponica Jonkonga 2
197 1826 Herb Switzerland Mougeot 6
198 1934 Herb Turkey Haldigan 1
199 1892 Herb Eastern Oregon NA 1
200 1897 Herb Western Washington 2
201 1892 Herb Idaho NA 3
202 1923 Herb British Columbia 4
203 1904 Herb British Columbia 5
204 1964 Herb Greenland 1
205 1915 Herb Vienna Nadbö Huen 1
206 1935 Herb Vienna Korb 2
207 1972 Herb Vienna Seipka 3
208 1971 Herb Vienna Seipka 4
209 1937 Herb Vienna Korb 5
210 1897 Herb Vienna Ozstereich 6
211 1923 Herb Vienna Steicrmark 7
212 1897 Herb Vi. Raxalpenplateau 8
213 1880 Herb Vienna Janglbauer 9
214 1902 Herb Vienna Ziesché 10
215 1932 Herb Oslo Sør- Trødelag 1
216 1916 Herb Oslo Birlivaay 2
217 1959 Herb Oslo Jämtland 3
218 1927 Herb Oslo Lapland 4
219 1966 Herb Os.Møre og Romsdal 5
220 1957 Herb Oslo Nordhal More 6
221 1907 Herb Oslo Gieranger 7
222 1910 Herb Oslo Opdal 8
223 1927 Herb Oslo Grane 9
224 1884 Herb Oslo Tromsö 10
225 1870 Herb Oslo Söderén 11
226 1949 Herb Oslo RYB 296 12
227 1968 Herb Oslo Gran 13
228 1978 Herb Oslo Lunner 14
229 1919 Herb Oslo Lom 15
230 1926 Herb Oslo Åseral 16
231 1935 Herb Oslo Nordfjord 17
232 1929 Herb Oslo Lavik 18
233 1934 Herb Oslo Nordfjord 19
234 1902 Herb Os. Skandalshorgen 20
235 1901 Herb Oslo Bygland 21
236 1966 Herb Oslo Buskerud 22
237 1995 Herb Oslo Buskerud 23
238 1942 Herb Oslo Krokan 24
239 1930 Herb Oslo Gauldalen 25
240 1948 Herb Oslo Lårdal 26
241 1986 Herb Oslo Buskerud 27
242 1991 Herb Oslo Hedmark 28
243 1900 Herb Oslo Tryssil 29
244 1947 Herb Oslo Alvdal 30

Appendix Ib

Figure I Principal components analysis with numbers (Appendix Ia) that indicate individual specimens.



Appendix II Sources of spores used for various experiments (5.2, 5.3) (CU = cultivated 96 or 97 gives the year of collection)

Table IIa *A. flexile*	**Bridge of Orchy BO**		**Glen Prosen GP**			**Ben Alder BA**		**Creag Meagaidh CM**		**Beinn Eibhinn BE**
treatment	BOAFy 96	BOAF5 97	GPAFL 96	GPAF CU97	GPAF2 97	BAAF E96	BAAF CU97	CMAF 97	BEAF 97	BEAF7 96
Germination						X				X
5 °C	X		X			X				X
10 °C	X		X			X				X
15 °C	X		X			X				X
20 °C	X		X			X				X
25 °C	X		X			X				X
30 °C	X		X			X				X
30°C +	X		X			X				X
Daylength										
24 Hrs	X		X							
18 Hrs	X		X							
12 Hrs	X		X							
6 Hrs	X		X							
Gametophyte growth										
No nystatin	X	X		X	X		X	X	X	X
pH3.8	X	X		X	X		X	X	X	X
pH 5.8	X	X		X	X		X	X	X	X
pH 7	X	X		X	X		X	X	X	X
-Mg	X	X		X	X		X	X	X	X
-Fe	X	X		X	X		X	X	X	X
-Ca	X	X		X	X		X	X	X	X
-P&K	X	X		X	X		X	X	X	X
25 C	X	X		X	X		X	X	X	
20 C	X	X		X	X		X	X	X	
15 C	X	X		X	X		X	X	X	
10 C	X	X		X	X		X	X	X	

Table IIb *A. distentifolium*	**Bridge of Orchy**		**Glen Doll**	**North west**		**Ben Alder**		**Creag Meagaidh**	**Beinn Eibhinn**	
treatment	BOAD1 97	BOAD1 96	GDAD2 96	Fuar Tholl 97	BAADC 96	BAADB 96	BAAD CU97	CMAD 97	BEAD 97	BEAD9 96
Germination										
5 °C		X	X		X		X			X
10 °C		X	X		X		X			X
15 °C		X	X		X		X			X
20 °C		X	X		X		X			X
25 °C		X	X		X		X			X
30 °C		X	X		X		X			X
30 °C +		X	X			X			X	
Daylength										
24 Hrs		X	X			X				X
18 Hrs		X	X			X				X
12 Hrs		X	X			X				X
6 Hrs		X	X			X				X
Gametophyte growth										
No nystatin	X	X	X	X		X		X	X	X
pH3.8	X	X	X	X		X		X	X	X
pH 5.8	X	X	X	X		X		X	X	X
pH 7	X	X	X	X		X		X	X	X
-Mg	X	X	X	X		X		X	X	X
-Fe	X	X	X	X		X		X	X	X
-Ca	X	X	X	X		X		X	X	X
-P&K	X	X	X	X		X		X	X	X
25 C	X	X	X			X	X	X	X	
20 C	X	X	X			X	X	X	X	
15 C	X	X	X			X	X	X	X	
10 C	X	X	X			X	X	X	X	

Appendix III Formulae for preparation of growth media (Dyer, 1979)

pH 3.8 was mixed by making stock solutions that were kept for up to 12 months in the cold room at 4 °C. From the stock solutions smaller quantities were taken for each batch mixed. pH 5.8 and 7.0 solutions were mixed as required and used fresh.

Stock solution A: 51 g l^{-1} of $MgSO_4 . 7H_2O$

12 g l^{-1} KNO_3

B: 1.7 g l^{-1} $FeCl_3 . 6 H_2O$

C: 144 g l^{-1} $Ca(NO_3)_2 . H_2O$

D: 25 g l^{-1} KH_2PO_4

E: Mycostatin 10 000 units ml^{-1}

10 ml of each of the stock solutions were mixed into 950 ml distilled water with 7 g l^{-1} and autoclaved for 40 minutes. The Nystatin (Mycostatin) was added only after the liquid had cooled below 60 °C.

Appendix IV Source of spores, number of gametophytes, and time taken to produce sporophytes in breeding experiments (6.2).

BO = Bridge of Orchy, BA = Ben Alder, GP = Glen Prosen, CM = Creag Meagaidh, GD = Glen Doll, BE = Beinn Eibhinn.

Table IVa Intergametophytic selfing, pairs of gametophytes, same parent

	number used	sporophytes	months taken
BOAF5	12	0	6
BAAF8	24	7	6
BOAD5	10	3	7
BOAD2	12	8	7

Table IVb Intergametophytic crossing, pairs of gametophytes, different parents

	number	sporophytes	months taken
GPAD0 x BAAD4	48	31	4
BOAD2 x BOAD5	12	8	7
BOAF5 x BOAF4	12	8	7
CMAF1 x BAAF8	47	12	6
GPAD0 x BAAF8	25	11	4
BAAD4 x CMAF1	25	4	4

Table IVc Intragametophytic selfing, single gametophytes

	number	sporophytes	months taken
BOAF5	12	3	7
BAAF8	23	8	6
BOAD2	11	4	6
BAAFG	100	19	6
BEAF7	100	20	6
BOAF6	100	44	6
BOAD2	100	23	6
BOAD7	100	31	6
GDAD2	100	50	6

Appendix V

Table V Gender of gametophytes grown on different media (6.2).
M = male, MF = male and/or female, O= neither

BO = Bridge of Orchy, BA = Ben Alder, GP = Glen Prosen, CM = Creag Meagaidh, GD = Glen Doll, BE = Beinn Eibhinn, F T = Fuar Tholl, CU = Cultivated plant.

	No N	pH 3.8	pH 5.8	pH 7	-A	-B	-C	-D
BAAD	M	M	M	M	O	O	M	O
BAAF	M	M F	M	M	M	M	M	M
F T AD	M F	M F	M	M	O	O	O	M F
CMAF	M	O	M	M	O	M	O	M F
CMAD	M	O	M	M	M	M	O	M
BEAF2	M	O	M	O	O	M	M	M F
BEAD1	M F	M	M	M	M	M	M	M
BEAF7	M F	M	M	M F	M	M	M F	M
BEAD9	M	M F	M	M	M	M F	M F	M
BOAFy	O	M	M	O	O	M	M	M
BOAD1 1997	O	M	M	O	O	M	M	M
BOAF5	O	O	M	O	O	M	M	M
BOAD1 1996	M	M	M	M	M	M	O	M
GPAFCU	M	M	M	M F	O	O	M F	M
GDAD2	M F	M F	M	M F	M F	M F	O	M F
GPAF2	O	O	M	M	M	O	M	O

Appendix VI: Preliminary analysis of isozyme markers in *A. distentifolium* and *A. flexile* by Johannes Vogel

In order to determine the taxonomic status of *A. flexile* and its relationships with *A. distentifolium* biochemical markers were used. Allozymes are a biparentally inherited co-dominant marker system which is well studied in plants. The number of isozymes (different forms of an enzyme encoded by different loci) is highly conserved in plants (Gottlieb 1981, 1982) and its applicability for fern biosystematics has been demonstrated in many studies (Werth *et al.* 1989; Soltis & Soltis 1989). The experiments and analysis were carried out in collaboration with the research team at the Botany Department of the Natural History Museum in London. The data from this work presented here are preliminary and will be the subject of future joint publications.

Methods

Allozyme electrophoresis has been carried out on *Athyrium* plants using established methods (Soltis *et al.* 1983; Haufler 1985; Wendel & Weeden 1989; Weeden & Wendel 1989). The following enzyme systems were informative and could be analysed for locus and allelic variation: Phosphoglucoisomerase (PGI, E.C. 5.3.1.9), 6 Phosphogluconate dehydrogenase (6 PGD, E.C. 1.1.1.44), Isocitrate dehydrogenase (IDH, E.C. 1.4.1.42), Hexokinase (HEX, E.C. 2.7.1.1), Malate dehydrogenase (MDH, E.C. 1.1.1.37), Diaphorase (DIA, E.C. 1.6.99), Aconitase (ACON, E.C. 4.2.1.3), Leucine aminopeptidase (LAP, E.C 3.4.11.1), Shikimate dehydrogenase (SkDH, E.C 1.1.1.25), Triose-phosphate isomerase (TPI, E.C. 5.3.1.1), Phosphoglucomutase (PGM, E.C.5.4.2.2), Asparate amino-transferase (AAT, E.C. 2.6.1.1) and Glutamate dehydrogenase (GDH, E.C. 1.4.1.2). Enzyme systems were resolved on a combination of several gel/buffer systems using 12.8% starch gels. The enzyme systems IDH, MDH, 6-PGD, HEX, SkDH, ACON, GDH were resolved with morpholine-citrate buffers over the pH range of 6.4 - 7.4 (Wendel & Weeden, 1989), while LAP, PGI, PGM, TPI, AAT, DIA were resolved on a modified System 7 (Soltis *et al.* 1983) over the pH range of 8.3 - 9.3 (Table A6.1). Band homologies were determined by running samples side-by-side on the same gel. Allelic variants within loci were distinguished from products of different loci by assuming that the enzymes in *Athyrium* conform to established models of organellar compartmentalisation (Gastony & Darrow, 1983, Weeden & Wendel, 1989) and by analysing patterns of bands from natural, interspecific hybrids (*Athyrium* x

reichsteinii). The most anodally migrating locus was labelled "1", and alleles were designated alphabetically.

The data were transferred into a Nexus-file and computed with the programme Genetic Data Analysis (GDA) written by Paul Lewis, University of New Mexico. This programme has been used to obtain a measure of genetic distance and identity between the populations using UPGMA cluster analysis after Nei (1978) (Table 6.3.5, Figure VI.2). This analysis takes into account the presence or absence and the frequency of alleles.

In this study, the allozyme data were analysed to test the species identity of *A. flexile* in relation to *A. distentifolium* and to utilise the data for biosystematic studies. Therefore, the population substructuring and clustering of small groups of ferns, as recognised in the field, has been mostly ignored in this analysis, and will be fully analysed later for a population genetic study. For the present study, all plants determined in the field either as *A. flexile* or *A. distentifolium* at a locality, i.e. Ben Alder, have been treated as one population. Therefore, at Ben Alder the two populations entered into the data set were BA-**D** for all *A. distentifolium* and BA-**F** for all *A. flexile*.

The final analysis and manipulation of data to investigate the population dynamics, migration rates and gene flow, breeding systems and population substructuring for *Athyrium distentifolium* has not been completed.

Results

Thirteen enzyme systems with 21 loci could be analysed. Of these allelic variation was recorded for nine loci. Of the variable systems PGI had three alleles and two alleles were recorded for 6-PGD-1, PGM-2, HEX, AAT-1, MDH-1, MDH-2, MDH-4 and GDH. Variation in PGI-2, 6-PGD-1, PGM-2, HEX, AAT-1 and MDH-4 was widespread and present in most populations. The allele *B* in MDH-2 was private to the population from the Pyrenees. The allele *B* in MDH-1 was only found in the population from the Alps, the Pyrenees and Ben Alder. The genetically most depauperate population was Glen Prosen with variation only in PGI-2, and here only the alleles *A* and *B*. The variation in GDH was not always well resolved and has been excluded from the analysis.

At all 21 loci, variable or none variable, the alleles present in *A. flexile* were also present in *A. distentifolium*. This results in the extremely high genetic identity between the various populations of *A distentifolium* and *A flexile* from Scotland and continental Europe. In contrast, only a few alleles were shared between *A. filix-femina* and the *A. distentifolium/flexile* - complex.

Albeit being based on a preliminary analysis the UPGMA phenogram (Figure 6.3.5) and genetic distance matrix (Table VI.2) show interesting trends and results:

i) the grouping of populations is mostly after localities and not after taxa. This means that the plants of *A. distentifolium* from Ben Alder are more similar to the plants of *A. flexile* from Ben Alder than to, perhaps, the *A. distentifolium* from Glen Prosen. The grouping by locality, and not by taxon, is true for all sites where both taxa are extant, i.e. Ben Alder, Glen Prosen, Beinn Eibhinn, Bridge of Orchy and Creag Meagaidh.

ii) the UPGMA phenogram distinguished three major groupings:

1. Pyrenees (probably due to the private B allele in MDH-2)
2. A group of Scottish populations containing all populations with extant and joint occurrences of both *A. distentifolium* and *A. flexile*. This group also contains the pure *A. distentifolium* populations from Craig an Duine.

3. A group of populations from the Alps (Switzerland), the Erzgebirge (central Europe, Germany, west of Dresden) and some populations from Scotland, from two

localities in Easter Ross above Loch a Mhadaidh, Ben Wyvis and Corrie Garbhlach in the Cairngorms.

The small subset population *C* of *A. distentifolium* from Glen Prosen (GP-D-C) which was included by the programme in this group is based on fact that this population of four plants is monomorphic for the *B* allele in 6-PGD-1, while all other plants from Glen Prosen are monomorphic for the *A* allele.

Discussion

The data set presented here is one of the more comprehensive for pteridophytes and covers populations from a large geographic area. The extremely low genetic distance and high genetic identity between populations of *A. distentifolium* and *A. flexile* shown in this data set is in line with reported data on genetic identity within fern species (Soltis & Soltis 1989). The fact that *A. flexile* shares the same alleles with *A. distentifolium* at all loci, and the results from the analysis after Nei (1978), support the hypothesis that both taxa represent one species.

Furthermore, at no locality where both taxa were present, did either taxon possess any private alleles. In fact, at all sites, where the two occurred together, all allelic variation, and also the frequencies of alleles, were shared. This would strongly support the hypothesis that at each site *A. distentifolium* and *A. flexile* exchange propagules and form an interbreeding population. This is furthermore supported by the result from the UPGMA cluster analysis which, after taking presence/absence as well as the frequencies of alleles into account, groups populations after localities, and not after taxa. This can be interpreted as evidence for the fact that in each corrie *A. distentifolium* and *A. flexile* form a distinct interbreeding population, somewhat isolated from other populations.

The surprising result of the clustering of all populations of *A. distentifolium/flexile* into a group distinct from other "pure" *A. distentifolium* stands from continental Europe and elsewhere in Scotland warrants further investigation with more populations from mainland Europe and Scandinavia. However, if this result can be maintained, it can be taken as indication for a special adaptation of a race of *A. distentifolium* in Scotland which might be isolated from other populations further afield. One of the special adaptations would then be manifested in the distinct morphological type of *A. flexile*. On the other hand, if the grouping stays constant with no continental populations falling into the "*flexile*-group", the presence of

"pure" *A. distentifolium* populations from Scotland outside the "*flexile*-group" would indicate either multiple colonisations of Scotland by *A. distentifolium* in the past or a differentiation of populations since the retreat of the glaciers, with some remaining genetically close to mainland European populations.

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Table VI.1: Electrophoresis of enzymes in *Athyrium distentifolium/flexile*. Number of loci scored and analysed and number of loci which are not resolved satisfactorily in parentheses. Abbreviations of electrophoretic systems, all systems on 12.8% starch: M-C = morpholine citrate, pH varied between 6.4 - 7.4; L-B = Lithium-borate system 7 after Soltis *et al.* (1983), pH varied between 8.3 - 9.3.

Enzyme system	Abbreviation	Electrophoretic system used	Number of loci scored
Aconitase	ACN	M-C	2
Asparate amino-transferase	AAT	L-B	1 (1-2)
Diaphorase	DIA	L-B	2
Glutamate dehydrogenase	GDH	M-C	(1)
Hexokinase	HEX	M-C	1
Isocitrate dehydrogenase	IDH	M-C	1
Leucine aminopeptidase	LAP	L-B	1
Malate dehydrogenase	MDH	M-C	4
Phosphoglucomutase	PGM	L-B	2
6-Phosphogluconate dehydrogenase	6-PGD	M-C	2
Phosphoglucose isomerase	PGI	L-B	2
Shikimate dehydrogenase	SKdH	M-C	1
Triose-phosphate isomerase	TPI	L-B	2
Total: 13			21 (2)

Table VI.2 Distances/identity measures based on 8 loci

Matrix has 23 populations Cluster = UPGMA Nei (1978) identity used above diagonal. Nei (1978) distance used below diagonal

	Alps	BA-D-A	BA-F-B	BO-D-A	BO-F-A	Pyren	Erzge	C-an-D-1	C-an-D-2	East-Ross	850-L-a-M	600-L-a-M	CM-F-A	CM-D-E	CG-D	BE-D-A	BE-F-C	LB-D	GE-D-A+F	GP-D-A	GP-D-C	GP-F-A	GP-F-B
Alps	0	0.907	0.913	0.907	0.911	0.874	0.973	0.917	0.892	0.966	0.96	0.911	0.945	0.958	0.942	0.954	0.957	0.922	0.925	0.878	0.921	0.88	0.855
BA-D-A	0.098	0	1.001	0.99	0.984	0.867	0.874	0.918	0.966	0.908	0.874	0.788	0.929	0.925	0.865	0.951	0.958	0.989	0.947	0.941	0.823	0.932	0.879
BA-F-B	0.091	-0.001	0	0.988	0.981	0.871	0.878	0.915	0.963	0.909	0.875	0.786	0.93	0.926	0.868	0.95	0.959	0.987	0.947	0.943	0.828	0.935	0.885
BO-D-A	0.098	0.01	0.012	0	0.995	0.852	0.896	0.912	0.957	0.921	0.899	0.82	0.926	0.941	0.881	0.967	0.978	0.997	0.957	0.913	0.857	0.894	0.816
BO-F-A	0.094	0.016	0.019	0.005	0	0.857	0.916	0.935	0.967	0.935	0.894	0.863	0.933	0.96	0.898	0.976	0.981	0.996	0.972	0.935	0.878	0.914	0.831
Pyrenees	0.135	0.143	0.138	0.16	0.155	0	0.865	0.873	0.872	0.885	0.832	0.795	0.885	0.879	0.857	0.879	0.877	0.87	0.886	0.874	0.807	0.871	0.83
Erzgebirge	0.027	0.135	0.13	0.11	0.087	0.145	0	0.925	0.895	0.979	0.954	0.955	0.944	0.989	0.978	0.97	0.968	0.92	0.959	0.883	0.972	0.869	0.804
Craig-aD-1	0.087	0.086	0.089	0.092	0.068	0.135	0.078	0	0.984	0.981	0.917	0.918	0.963	0.969	0.897	0.967	0.95	0.944	0.962	0.945	0.86	0.932	0.869
Craig-aD-2	0.115	0.035	0.038	0.044	0.034	0.136	0.111	0.016	0	0.951	0.9	0.848	0.968	0.96	0.888	0.972	0.963	0.977	0.978	0.973	0.845	0.958	0.89
East-Ross	0.034	0.096	0.096	0.082	0.067	0.122	0.021	0.02	0.051	0	0.963	0.947	0.961	0.987	0.937	0.982	0.97	0.95	0.964	0.903	0.914	0.888	0.822
850-L-a-M	0.041	0.135	0.133	0.106	0.112	0.183	0.047	0.087	0.106	0.038	0	0.908	0.972	0.962	0.956	0.969	0.969	0.911	0.938	0.852	0.939	0.842	0.792
600-L-a-M	0.093	0.238	0.24	0.199	0.147	0.229	0.046	0.085	0.165	0.055	0.097	0	0.891	0.947	0.916	0.921	0.905	0.846	0.898	0.823	0.924	0.805	0.731
CM-F-A	0.056	0.074	0.073	0.077	0.07	0.122	0.058	0.038	0.032	0.039	0.029	0.116	0	0.982	0.966	0.985	0.976	0.943	0.982	0.958	0.931	0.953	0.91
CM-D-E	0.043	0.078	0.077	0.061	0.041	0.129	0.011	0.032	0.041	0.013	0.038	0.055	0.018	0	0.973	1	0.994	0.962	0.995	0.942	0.964	0.925	0.852
CG-D	0.06	0.145	0.142	0.126	0.108	0.155	0.022	0.109	0.118	0.065	0.045	0.087	0.034	0.028	0	0.959	0.953	0.894	0.96	0.901	0.977	0.893	0.844
BE-D-A	0.047	0.05	0.051	0.033	0.024	0.129	0.031	0.034	0.028	0.018	0.032	0.082	0.015	0	0.042	0	1.003	0.981	0.995	0.94	0.945	0.923	0.848
BE-F-C	0.044	0.043	0.042	0.022	0.019	0.132	0.032	0.051	0.038	0.025	0.032	0.099	0.024	0.006	0.048	-0.003	0	0.988	0.99	0.927	0.944	0.909	0.832
LB-D	0.081	0.011	0.013	0.003	0.004	0.139	0.083	0.058	0.024	0.052	0.093	0.167	0.059	0.039	0.112	0.019	0.013	0	0.974	0.933	0.871	0.913	0.834
GE-D-A+F	0.078	0.054	0.054	0.044	0.028	0.121	0.042	0.039	0.022	0.037	0.064	0.108	0.018	0.005	0.04	0.005	0.01	0.027	0	0.965	0.939	0.947	0.87
GP-D-A	0.13	0.061	0.059	0.091	0.067	0.135	0.124	0.057	0.028	0.102	0.161	0.195	0.043	0.06	0.105	0.062	0.076	0.07	0.036	0	0.862	0.998	0.955
GP-D-C	0.082	0.195	0.189	0.155	0.13	0.215	0.028	0.151	0.168	0.09	0.063	0.079	0.071	0.037	0.023	0.056	0.058	0.139	0.063	0.149	0	0.846	0.778
GP-F-A	0.128	0.07	0.067	0.112	0.09	0.138	0.14	0.071	0.043	0.119	0.171	0.217	0.048	0.078	0.113	0.08	0.096	0.091	0.055	0.002	0.168	0	0.978
GP-F-B	0.156	0.129	0.122	0.204	0.185	0.186	0.218	0.141	0.117	0.196	0.234	0.313	0.094	0.16	0.17	0.165	0.184	0.182	0.14	0.046	0.251	0.023	0

NOTES AND OBSERVATIONS

Larva of *Autographa gamma* (Linnaeus) (Lepidoptera: Noctuidae) feeding in the wild on *Athyrium distentifolium*

On 18 August 1996 a noctuid larva was found feeding on the fronds of the Alpine Lady-fern, *Athyrium distentifolium* Tausch ex Opiz, in a corrie above Crannach near Bridge of Orchy (Argyll) at an altitude of 850 m. In captivity it was offered the Common Lady-fern, *A. filix-femina* (L.) Roth., but only resumed eating when offered its original foodplant, *A. distentifolium*. It pupated nearly three weeks later and emerged on 7 October 1996 as *Autographa gamma* (Linnaeus). The adult is a particularly dark specimen. Skinner (1984, *Colour identification Guide to Moths of the British Isles*: 150) records that the larva will feed on most low plants. This record is of especial interest as few species eat ferns.

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Photo: Heather McHaffie

Polypodium flexile Moore

One of the original Glen Prosen plants collected by James Backhouse in 1852.

GLEN PROSEN REVISITED*

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On the 24th of July 1852 three botanists came over the hill from Corrie Fiadh at the head of Glen Clova. There was James Backhouse, well known for his nursery in York. He was accompanied by his son, also called James, and a friend, Thomas Westcombe. They had spent several days in the area looking at alpine plants. Of especial interest to them was a species of lady fern that had only recently been recognised in this country as distinct from *Athyrium filix-femina*. At that time it was called *Polypodium alpestre*, the alpine lady fern, now *Athyrium distentifolium* Tausch ex Opiz (Plate 5).

As they came round the slopes of Maire they came to a rocky outcrop above the Maire Burn. Here they found more *P. alpestre* looking superficially very similar to *Athyrium filix-femina*. But on examining the back of the frond the circular sori were clearly visible, demonstrating its classification as a polypody. These were very different from the j-shaped sori of *A. filix-femina* and almost entirely lacked indusia. As they looked closer it became apparent that there was also something else, a smaller, narrow-fronded form, quite unlike the usual *P. alpestre* which they had been collecting for several days (Plate 6). As James Backhouse (senior) later wrote: *A remarkable variety, with deflexed pinnae, was met with in one place in Glen Prosen.* (Backhouse, 1852).

They found the discovery of sufficient interest to merit the collection of a substantial number of robust, fertile fronds. These were taken back to York and were doubtless the subject of much scrutiny and discussion. Letters would have been written, and in May 1853 the Backhouses sent specimens of both types to Edward Newman who thus addressed the Phytologist Club:

> *The President observed that since he had the pleasure of inviting attention to the occurrence, in Scotland, of a fern previously unrecorded as British, several very ardent and most acute botanists had searched the districts indicated, and with complete success. The result, however, was the discovery of not a* single *species alone, but of two. Through the kindness of Mr Backhouse, he had had the opportunity of examining an extensive and very beautiful series of each of these; and although in this early stage of the inquiry he by no means wished to do more than indicate the obvious distinguishing characters, he considered it due to his friends to communicate to the public the results of their researches.*
>
> (Newman, 1853)

Newman decided at the same time that a more appropriate generic name for the two would be *Pseudathyrium*. Both *Pseudathyrium* and *Polypodium* were used throughout

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the 19th century until eventually *Athyrium* became more generally used into the 20th century, and *alpestre* has been replaced by the current *distentifolium*. He provisionally called his new fern *Pseudathyrium flexile*, but as it has never been found anywhere else within the circumpolar range of *A. distentifolium*, it has retained the specific name. Its common name has recently been changed from the flexile lady fern to Newman's lady fern.

Newman gave *flexile* its name from the tendency of the stout stipe to bend sharply near the base so the fronds are held flat against the ground. He gave a detailed description of both *P. alpestre* and *P. flexile*. The blade of the smaller *flexile* is very narrow and the pinnules are less frilly. If the fronds are only partially fertile, then *flexile* is usually only fertile at the base, and *distentifolium* (or *alpestre*) is only fertile at the tip. This tendency to opposites is continued in the more congested nature of the deflexed lower pinnae of *flexile*, contrasted with widely spaced basal pinnae in *distentifolium*. Conversely, *flexile* has widely spaced terminal pinnae, while in *distentifolium* they are more crowded.

Newman was confident that the fern was sufficiently distinct and over-ruled any reservations by those who had originally found it: *Mr Westcombe and Mr Backhouse entirely abandon the idea of it being a form of* alpestre, *although there can be no doubt, we have seen that this idea did present itself to both of them at the moment of finding it, possibly because they were totally unprepared for the occurrence of a second new fern on ground for so many years trodden by our Scottish friends in their herborising excursions.* (Newman, 1854). In 1859 Thomas Moore expressed some reservations: *It is certainly a very distinct variety, and very constant, probably a variety rather than a species, this moreover being the view adopted by its discoverer Mr Backhouse, who writes: Dissimilar as it is from* P. alpestre, *I shall continue doubtful of its specific difference if it does not turn up in other places.* (Moore, 1859).

In due course other localities were reported. In 1867 it was first found at Ben Alder, which has the largest population of any known site. It has been recorded, sometimes as only a single plant, right across the Central Highlands. There are records from Knoydart, Ben Nevis, Glen Spean, Bridge of Orchy, Glen Lyon, Beinn Eibhinn and several places in the Cairngorms, Lochnagar and Caenlochan.

The locality at Glen Prosen was visited with diminishing frequency towards the end of the 19th century, especially once the Ben Alder site had been discovered. The last known herbarium specimen of *P. flexile* was collected in 1894 and after that there is no further mention of it in the area. Cowan (1915) reports a Scottish Alpine Botanical Club excursion to Glen Prosen in 1914 but only mentions *P. alpestre*. Perhaps the plants had been over-collected, or the sheep and deer ate them into oblivion. Keen botanists concentrated on the basic rocks in Glen Clova where there was ample scope for a day spent botanising.

On the 27th of June 1995 David Ellis and I came over the hill from Corrie Fee. There was no time to go wandering off to look for woodsias, no deviating from the path to have a quick search for *Lycopodium annotinum*. Watched by a suspicious pair of eagles we purposefully climbed up to the watershed. It was the beginning of a long, hot summer. We scrunched across the lichen on the tundra-like plateau and came down into the head of Glen Prosen. For months I had been looking at maps of the area. I had read and re-read the sparse clues offered by the herbarium sheets: *Micaceous rocks at the head of Glen Prosen . . . . above the Maire Burn*. From my reading and limited experience I had definite ideas about a suitable habitat for *A. distentifolium*, and hence also for *flexile*. I was looking for a north, north-east or north-west facing corrie above at least 600 or 700 m. Only a limited range of species can survive the extended snow-cover found in suitable places at this altitude and

this gives the ferns a competitive advantage. Unfortunately, the map did not suggest anywhere very appropriate at all. The best I could find at more than 600 m was the South Craig with an aspect to the east-north-east.

As we approached the Craig, walking in above the Maire Burn, a brood of young ptarmigan flew away, and their mother stayed to distract us. It was the right sort of place for ptarmigan, with good big boulders. A lush carpet of *Vaccinium myrtillus* (blaeberry or bilberry) swept right round them and up to the foot of the craigs, leaving no spaces for any ferns on the ground. The ledges on the craigs were mostly too accessible to grazing sheep or deer. With difficulty, I scrambled up a rock face to reach the only visible clumps of fern. The first was *Athyrium filix-femina*, with bluey-green fronds, unlike the yellow-green of *A. distentifolium*. I had a slug's-eye view of the sparse, narrow, darker scales at the base of the *A. filix-femina* stipes. Infertile *A. distentifolium* can often be distinguished by the broad, paler scales. Also, *A. distentifolium* pinnules are more triangular than *A. filix-femina* which tends to have parallel-sided pinnules. On the next ledge there was lot of *Dryopteris oreades*. Finally I did encounter some *A. distentifolium*, so I worked methodically up and down across the parts that were most difficult to get to, but with no sign of the absent *flexile*.

Eventually I crashed back down at the appointed time to meet my companion. I was hot, scratched and despondent. All that remained was the weary trek up the hill, the trudge all the way across the top in the baking sun, and the steep descent on the other side. In a misguided attempt to cut the corner, I suggested that we should go straight up the hill so as to omit two sides of the triangle we had previously followed.

It was extremely warm. I trailed behind up an impossibly steep slope of *Nardus stricta* (mat grass). Had I felt more my usual didactic self I could have said that just such a vegetation was typical of late winter snow-lie. However, on pausing yet again to look at the view with unseeing eyes, I did notice some large rocks with ferns in the middle. The bright yellow-green suggested *A. distentifolium* and it seemed a good excuse for a rest to go and look closer. Some of the rocks were flat enough to sit on, and yes, it was *distentifolium*. The largest clumps growing in the middle of the rocks had been grazed but the arrangement of the slabs was such that there were good gaps underneath, so I peered in. And there, at last, was what I was looking for. The same narrow frond that I had seen at Ben Alder, the same fertility pattern and the same distinctive habit. It was indeed the fern that Newman had called *Pseudathyrium flexile*.

The herbarium specimens of the Glen Prosen plants tended to have wider spaced pinnae than many of those from elsewhere, and these did also. On a warm, sunny, south-facing slope expansion was probably more rapid than in other sites. A quick survey round the rocks revealed more plants. Some, at the end of June, were nearly ready to shed their spores, although that was an exceptional season. After much taking of photographs I went on up the slope with strangely renewed vigour and happily skipped off across the skyline. Although I have since seen the fern in several other locations, mostly in far greater abundance, there was a distinct satisfaction in having re-found the original type locality.

Just over a year later I surveyed the trays of ferns in the university greenhouse with rather less jubilation. During the unusually dry summer of 1995 I had collected a wide range of spores in near perfect conditions. I had carefully nurtured the gametophytes and watched their development. There were now hundreds of young plants from Ben Alder, Glen Prosen, Creag Meagaidh, Bridge of Orchy and Glen Coe. The *flexile* plants had grown very quickly and some had produced spores within nine months of sowing. *A. distentifolium*, true to my previous experience, was less precocious although vegetatively larger. But all was not well. How could I have been so careless? There are always a few spores of other species which creep in as rogue plants, but to have nearly half of some trays with two

different types of ferns seemed to imply a disgraceful amount of contamination. Then a pattern began to emerge. On the whole, *flexile* spores produced *flexile* sporophytes. There were four *distentifolium* plants among 415 *flexiles*; very much the rogue-level one might expect. There were seven batches of *distentifolium* that were apparently pure although they were mostly rather small samples. But eleven sets of spores, collected in the field from apparently typical *distentifolium*, had produced anything from a quarter to one half *flexile* sporelings.

For some time I was puzzled by these ratios. Using Mendel's work to explain a possible one-gene mutation, the expectation would be one quarter pure *flexile*-type plants, one quarter pure *distentifolium*-type, and one half of the plants would look like *distentifolium* but have the potential to produce the *flexile* mutation. This would mean that three quarters of the sporophytes should look like *distentifolium*, but this was not the case. However, because these ferns were the result of mixed mating, albeit with spores from the same parent, there was the opportunity for either self or cross-fertilisation to occur and this could explain the ratios.

It is tempting to wonder about James Backhouse's experience in cultivating these ferns. The Backhouse Nursery had great expertise in growing a wide range of native and foreign species of ferns and Newman (1854) reported that both *flexile* and *alpestre* were growing freely in cultivation at York. If spores of *flexile* alone been propagated then they would have produced true progeny and its status would have been no further clarified. Only if spores from certain *A. distentifolium* populations like Glen Prosen were sown, would the two kinds of sporophytes have been produced. Even then, a mixed batch could have been the result of contamination, especially as they were probably collected into a vasculum where they would have been in close proximity. At that time, ideas on genetic variation had not been developed. In 1859 Moore referred to James Backhouse's misgivings about the status of *flexile* which Newman had so firmly proclaimed. Why, seven years after it was first found was there still some doubt over its identity? What, in the light of his extensive fern-growing experience did James Backhouse observe? He might not have had an explanation for the results, but what conclusions did he draw?

This article reports part of an investigation into the ecology of Scottish *Athyrium distentifolium* and *flexile*. Research into their relationship is continuing.

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Photo: H. McHaffie

Plate 5: ***Athyrium distentifolium*** with ***D. expansa*** (p 88)

Photo: H. McHaffie

Plate 6: ***Athyrium flexile*** (p 88)