The Ecology and Genetics of *Cirsium dissectum* (L.) Hill in the British Isles and Implications for its Conservation

by

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Dedication

For Col, Ness and Jack.

Abstract

Natasha de Vere

The Ecology and Genetics of *Cirsium dissectum* (L.) Hill in the British Isles and Implications for its Conservation

The aim of this study was to research the ecology and genetics of *Cirsium dissectum* and to discuss the implications of the results for its conservation. The ecology of *C. dissectum* was described through a review of the literature. Site characteristics, plant communities and reproductive biology were investigated by examining 22 populations throughout the British Isles. Microsatellite genetic markers were used to investigate levels of genetic diversity within and between these populations. Within populations, relationships between genetic diversity, population size, fitness and habitat quality (concentrating on soil nutrients and vegetation structure) were explored using multiple regression and structural equation modelling. Differentiation between populations was examined by comparing microsatellite markers with morphological traits and this was supplemented by a crossing experiment that investigated the effects of inbreeding and outbreeding.

This study showed that *C. dissectum* was a clonal species with a mixed mating system. Previous research had suggested that clonal propagation was the dominant form of reproduction but this study showed that sexual reproduction was important in this species, as levels of genotypic diversity were high. There were interactions between population size, genetic diversity, plant fitness and habitat quality. Smaller populations of *C. dissectum* had lower genetic diversity and this subsequently reduced plant fitness. Higher levels of bare soil and phosphorus were related to higher levels of genetic diversity; bare

soil may provide establishment gaps for seedlings and clonal offspring, while phosphorus may encourage flowering and/or seedling survival.

Populations of *C. dissectum* showed high levels of genetic differentiation and strong isolation by distance using microsatellite genetic markers. Both microsatellite genetic markers and morphological traits revealed geographical structuring between populations, but this was less pronounced using the morphological traits. Plants in Ireland showed higher levels of morphological differentiation compared to Britain. *C. dissectum* showed strong, early acting inbreeding depression when plants were selfed and a trend towards outbreeding depression when genetically distant populations were crossed.

Populations of *C. dissectum* should be conserved throughout the geographical range of the species in the British Isles. Sites should be managed so that habitat heterogeneity is maintained, enabling *C. dissectum* rosettes to flower and to maintain bare soil for seedling establishment. Habitat restoration should use seed collected from a number of local populations of the same habitat.

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Author's Declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Graduate Committee.

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Publications

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Presentations and conferences attended

- December 2002: British Ecological Society Annual Meeting, University of York.
- September 2003: British Ecological Society Annual Meeting, University of Manchester.
- December 2003: Population Genetics Group Meeting, University of Sussex.

- September 2005: British Ecological Society Annual Meeting. University of Hertfordshire. Poster presentation.
- April 2006: Ecological Genetics Group Annual Meeting. University of Aberystwyth. Oral presentation. **Awarded first prize for best student presentation**.
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Chapter 1

1 Introduction

1.1 The conservation of plant species

Habitat destruction, over-exploitation, alien invasive species and climate change threaten the survival of many species worldwide (Baillie *et al.* 2004). One fifth of all plant species are threatened with extinction and urgent action is needed in order to protect them (IUCN 2006).

The conservation of plant diversity depends on the conservation of individual plant species and their inherent genetic variation. Conservation strategies for plant species must be based on thorough scientific knowledge of species biology, of which an understanding of genetics is a key aspect (Smith & Waldren 2006). The environment within which a species is found, its demography and genetics all interact to determine its probability of extinction.

Habitat destruction reduces the size of plant populations and small populations are susceptible to demographic and genetic stochasticity (Matthies *et al.* 2004). Demographic stochasticity is caused by chance realizations of individual probabilities of death and reproduction in a population; individual events tend to average out in large populations but

in small populations, chance fluctuations are more likely to lead to population extinction (Lande 1993). Small populations can also suffer from loss of genetic diversity and increases in inbreeding (Ellstrand & Elam 1993). Loss of genetic diversity may reduce the evolutionary potential of a species (Barrett & Kohn 1991), whilst inbreeding redistributes alleles from heterozygous to homozygous combinations and can lead to inbreeding depression through the phenotypical expression of harmful, or less fit, recessive alleles (Edmands 2007).

Environmental stochasticity and random catastrophes can also lead to extinction and these factors can affect populations of all sizes (Lande 1993). Habitat quality is an important factor in population viability and persistence but few scientific studies have examined interactions between habitat quality, population size, genetic factors and plant performance (Vergeer *et al.* 2003). Ouborg *et al.* (2006) describe conservation genetics and habitat factors as different paradigms within conservation biology and research tends to concentrate on one area or the other. In order to understand the effects of habitat destruction and modification on plant species, a complete understanding is needed of the inter-relationships between habitat quality, population size, genetic diversity and plant fitness, and these need to be placed within the context of the species' ecology.

To conserve plant species effectively, the following questions must be answered and this is possible through an understanding of species ecology and genetics.

a) Why is the species threatened?

A species may be threatened due to many factors such as habitat destruction, over-exploitation, or competition with invasive species but the impact of these external factors depends on the ecology of the species. Analysis of population dynamics can reveal "bottlenecks" in the species life-cycle that makes it susceptible (Jongejans *et al.* 2006b). Genetic analysis can resolve key features of a species' biology such as

dispersal (Ouburg *et al.* 1999) or mating system (Frankham *et al.* 2002) that will affect how it reacts to external pressures.

b) Which populations are conservation priorities?

Limited resources for conservation mean that it is impossible to protect all of the populations of a species and in this situation, conserving the maximum amount of genetic variation is a priority (Hamrick *et al.* 1991). Genetic analysis can reveal how genetic variation is partitioned within and between populations. If populations are highly differentiated, then more will need to be conserved to maintain the same proportion of genetic diversity (Hamrick *et al.* 1991).

c) How should the species' habitat be managed?

Many species depend on human mediated management for their survival and this is especially true within the British Isles, where nearly all of the original climax vegetation has been lost (Rackham 1995). Examining interactions between habitat factors, genetic diversity and fitness can help to suggest the habitat conditions that will optimize species survival.

d) How do you restore a species to an area where it has become extinct?

If a species or habitat has been lost from large areas, it may be necessary to translocate organisms in order to increase their distribution or to re-create habitats, and this requires a decision on the best source of organisms (Gilbert & Anderson 1998). The key considerations are whether to use local provenance or to maximize genetic diversity. Local populations may be better adapted, but if they are small, they may suffer from inbreeding depression. If populations are sourced from further away, then outbreeding depression may occur (Vergeer *et al.* 2004). Genetic analysis can

reveal how differentiated populations are and the effects of inbreeding and outbreeding can be investigated experimentally.

1.2 Study species: *Cirsium dissectum*

The overall aim of this thesis is to investigate the ecology and genetics of *Cirsium dissectum* (meadow thistle), in order to inform its conservation and to contribute to the discussion on plant species conservation.

C. dissectum is a rhizomatous herb found in wet, nutrient-poor, semi-natural grasslands in northwest Europe. It is endangered in Germany and the Netherlands (Jansen *et al.* 1996; Buck-Sorlin 1993; Buck-Sorlin & Weeda 2000; Jongejans 2004; van den Berg *et al.* 2005) and has declined in all of the countries within which it is found (Institut floristique Franco-Belge 1995; Preston *et al.* 2002; Hackney 1992). In Britain, it is listed as of "Least Concern" by Cheffings *et al.* (2005) but included as a species where there is a reasonable chance that Britain holds more than 25% of the European population. This indicates that Britain has an international responsibility to conserve this species. The relevance of *C. dissectum* to Britain is suggested by its synonym *C. anglicum* and common name in France, "cirse Anglais", and Germany, "Englischen kratzdistel", the English thistle.

C. dissectum is not a rare species in that it has always had a very limited distribution; although specialised in its habitat requirements, it can be abundant in suitable conditions (Preston *et al.* 2002). Instead, it is an example of a species that is characteristic of semi-natural grasslands, that in Europe, were traditionally managed through extensive grazing, burning and hay cutting. It is therefore a casuality of the changes in traditional farming practice that have led to losses in all types of semi-natural, oligotrophic grasslands

(HMSO 1995). *C. dissectum* is an example of a "new rare" (*sensu* Huenneke 1991); a species that has declined relatively recently through human influences. The effect of small population size and isolation may be different in these "new rare" species relative to naturally rare plants (Oostermeijer *et al.* 2003; Hooftman *et al.* 2004).

Changes in agricultural practices have led to current declines in species such as *C. dissectum* but the next major problem to be faced is climate change. In order to adapt to global warming, species and habitats need to shift distribution and this will not be possible for many species found in small and fragmented habitats within a sea of urban development and high intensity agriculture. *C. dissectum* has an Oceanic Temperate distribution (Preston & Hill 1997), which means that it depends on a warm, moist climate and may therefore be particularly vulnerable to climate change.

C. dissectum is a key species of European Cirsio-Molinietum grasslands, characterised in Britain as M24 (*Molinia caerulea – Cirsium dissectum* fen meadow) within the National Vegetation Classification (Rodwell 1991). Cirsio-Molinietum grasslands have declined throughout Europe and conservation strategies have been put in place to correctly manage the areas remaining and to restore the habitat to areas where it has been lost (Berendse *et al.* 1992; Jansen & Roelofs 1996; Jansen *et al.* 1996; Beltman *et al.* 2001). The other constant species within this community, *Molinia caerulea*, *Potentilla erecta*, *Succissa pratensis*, *Lotus pedunculatus* and *Carex panicea*, have broader habitat requirements than *C. dissectum* (Grime *et al.* 1990; Stace 1997; Preston *et al.* 2002). *C. dissectum* is therefore a useful indicator species for this community; if it is thriving, then the conditions are likely to be correct for the community as a whole (Ross 1999).

Investigating the ecology and genetics of *C. dissectum* will therefore help to conserve a declining species that Britain has an international responsibility to protect. Information on the best way to manage and restore *C. dissectum* will be of relevance for

the conservation of Cirsio-Molinietum grasslands and will also provide a useful case study into the ecology of a "new rare" species.

To establish the starting point for this study, a thorough review of the existing literature on *C. dissectum* is required. The following review provides a description of *C. dissectum* and investigates the taxonomy, distribution, habitat, response to biotic and abiotic factors, reproduction, genetics and conservation of this species.

1.3 Review of *Cirsium dissectum*

1.3.1 Description

Cirsium dissectum is a perennial herb with short obliquely ascending stock, cylindrical roots and rhizomes up to 40 cm long. The basal-rosette leaves are elliptical-lanceolate, long-stalked, sinuate-toothed or slightly pinnatifid. Leaves are slightly hairy above and whitish-cottony beneath with the margins bearing soft prickles, which are longest on the teeth or lobes. The flowering stem is erect, usually simple, terete, cottony and unwinged. There are usually a few small bract-like leaves above the middle, which are like the basal leaves but oblong-lanceolate and semi-amplexicaul with basal auricles. Capitula are usually solitary with an ovoid, purplish cottony involucre, with bracts that are lanceolate and appressed, the outer bracts are spine-tipped and the inner acuminate. Flowers are magenta-purple and hermaphrodite (Clapham *et al.* 1987; Stace 1997) (**Plate 1.1**).

Plate 1.1 *Cirsium dissectum.* Left: capitulum (Kenfig 08/06/02). Right: vegetative rosettes (Lough Talt 27/05/03).

1.3.2 Taxonomy

The genus *Cirsium* is composed of over 250 species distributed in the northern hemisphere, spanning subtropical to boreal latitudes. Its occurrence in the southern hemisphere is considered to be non-native. The highest species diversity is concentrated in the mountains of Southern Europe and in the Caucasus Mountains (Bureš *et al.* 2004). Tutin *et al.* (1976) recognised 60 species in Europe and there are eight native and two naturalised species in the British Isles (Stace 1997).

Tutin *et al.* (1976) placed *C. dissectum* within the *C. tuberosum* group, along with *C. tuberosum* and *C. filipendulum.* Within the British Isles, *C. filipendulum* is not found, and *C. dissectum* is readily distinguishable from *C. tuberosum* by its less pinnatifid leaves and long rhizomes, and absence of tuberous roots. All three species occur in Germany, but not in the same sites (Hegi 1966). In France, the three species can be difficult to distinguish and Tutin *et al.* (1976) considered that they could probably be treated as subspecies. Rouy (1905) recognised four forms of *C. anglicum* (synonym of *C. dissectum*): f. *typicum* with regular toothed leaves; f. *angustifolium* with almost linear leaves; f. *dissectum* with

irregularly incised or lobed leaves, being more robust than the previous two forms, and f. *ambiguum* with very pinnatifid leaves and strong growth, often with two or three stems. Hegi (1966) also described high levels of variation within the dissection of the leaves but in the British Isles no variants have been recognised (Sell & Murrell 2006).

1.3.3 Distribution

Cirsium dissectum can be described as an Oceanic West European (Matthews 1955) or Oceanic Temperate (Preston & Hill 1997) plant, concentrated in the British Isles in S.W. England, S. Wales and W. Ireland (**Figure 1.1**). It is found frequently in Devon, Dorset and Hampshire (particularly the New Forest) and in South Wales. It becomes patchy further north and east, and has declined greatly since 1930 (Preston *et al.* 2002). It is apparently extinct in eight vice-counties in eastern England (Stace *et al.* 2003) and has been lost from many localities elsewhere, although counties such as Oxfordshire and Berkshire still have a number of sites. The fens of Norfolk and to a lesser extent Suffolk still represent a stronghold. It becomes sporadic as it extends northwards but it has a number of sites in Yorkshire, north to Roxby, 54° 32' N. In Scotland, it is found only on Islay and S.E. Jura and the adjacent mainland of Kintyre, where it may have colonised naturally from N. Ireland or possibly been introduced. *C. dissectum* is frequent throughout western Ireland, but it is rare and declining in the north-east (Hackney 1992). From Britain, it extends eastwards as far as the Netherlands (Rossenaar & Groen 2003) and has a limited distribution in Belgium (Van Rompaey & Delvosalle 1972) and N.W. Germany (Haeupler & Schonfelder 1989) (**Figure 1.2**). It extends southwards through France, being relatively common (at least formerly) throughout the west, north and centre of the country (Bonnier 1851-1922). In Germany, 57% of populations have become extinct since 1930 (Buck-Sorlin 1993) and strong declines have also occurred in the Netherlands (Rossenaar & Groen 2003) and in eastern sites in northern France (Institut floristique Franco-Belge 1995).

Tutin *et al.* (1976) and Hegi (1966) list *C. dissectum* from Spain but such records are regarded by Bolòs & Vigo (1995) as almost certainly errors. Tutin *et al.* (1976) described its occurrence in Italy as doubtful and reports of its presence have not been confirmed (Fiori 1969; Pignatti 1982). It is naturalised in Hungary and Norway (Tutin *et al.* 1976).

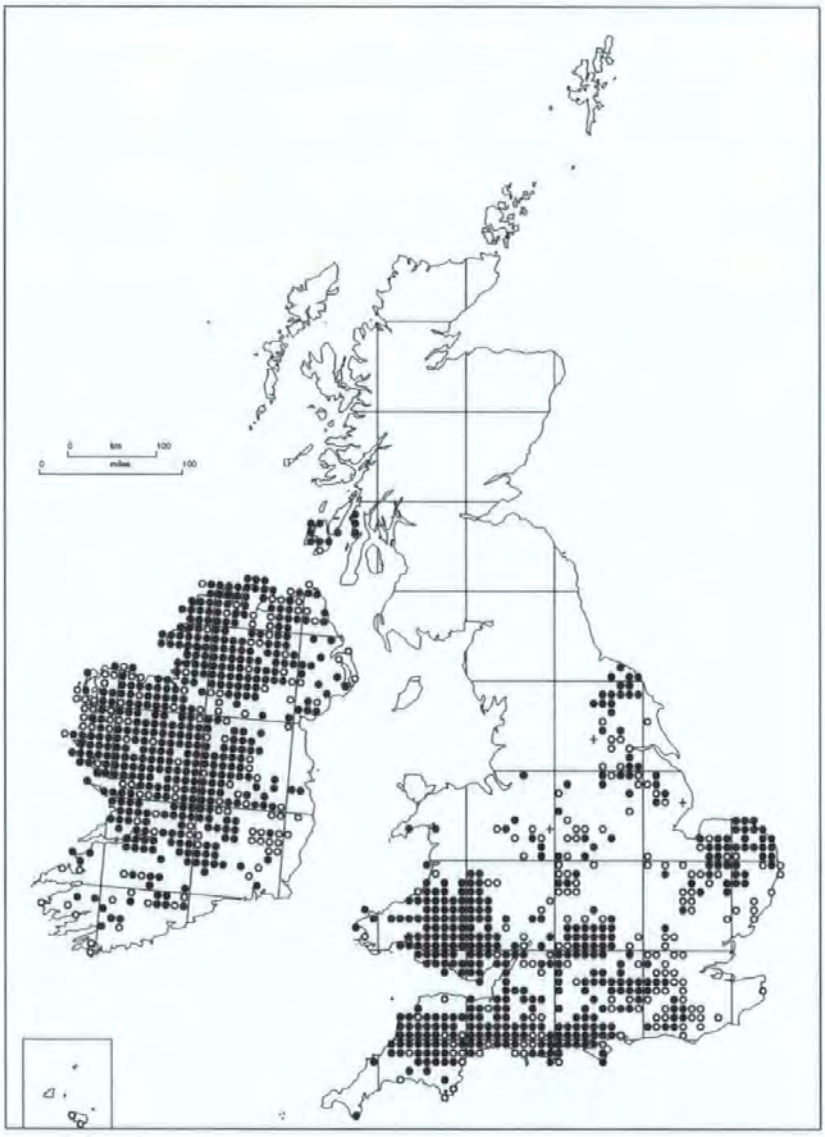


Figure 1.1 The distribution of *Cirsium dissectum* in the British Isles. (Each dot represents at least one record in a 10 km square of the National Grid. Native: (•) 1970 onwards, (o) pre-1970, (+) introduced. Mapped by H.R. Arnold, using Dr A. Morton's DMAP software).

Figure 1.2 European range of *Cirsium dissectum* (black shading) based on records in the literature (Tutin *et al.* 1976; Hegi 1966; Meusel *et al.* 1965; Bonnier 1851-1922; Haeupler & Schonfelder 1989; Preston *et al.* 2002; Van Rompaey & Delvosalle 1972. Squares represent countries where it is naturalised).

1.3.4 Habitat

1.3.4.1 *Climate and topography*

Cirsium dissectum has an Atlantic distribution, with Ellenberg (1988) classifying it as having a continentality value of 1, which indicates an extremely oceanic species. In Britain, however, it is also found in the warmer south-east, indicating that it has an Oceanic rather than Hyperoceanic distribution (Preston & Hill 1999). Buck-Sorlin (1993) states that the distribution in northwest Germany is determined by three climatic factors: a mean temperature in January greater than 0 °C, an annual fluctuation of mean temperature less than 16 °C and annual precipitation between 600 - 800 mm.

C. dissectum is found in sites that are permanently damp and can be found in sites with standing water during the winter months. This corresponds with an Ellenberg water value (recalibrated for British plants) of 8, intermediate between a damp and a wet site

indicator (Hill *et al.* 1999). It is a lowland species; the highest altitude recorded is 500 m in County Sligo (Preston *et al.* 2002).

1.3.4.2 *Substratum*

In the British Isles, *C. dissectum* is a characteristic species of rhos pasture. These are wet grasslands occurring on acidic to neutral soils comprising a mixture of fen meadow, rush pasture and wet heath often occurring in a mosaic. In Wales, these habitats are situated over a wide range of strata, ranging from sedimentary and igneous Lower Palaeozoic rocks, to Old Red Sandstone, Coal Measures and Carboniferous Limestone of Upper Palaezoic age (Blackstock *et al.* 1998). In Devon and Cornwall, rhos pastures are characteristically found on the Culm Measures, sandstones and shales of late Carboniferous age (Durrance & Lamming 1982) which cover much of mid-Devon through to north-east Cornwall. Soil types include poorly draining surface-water gleys with non-humose, humose or peaty topsoils and peats (Blackstock *et al.* 1998; Ross 1999). *C. dissectum* also occurs on sand dune slacks on typical sand-pararendzinas (Ross 1999). In Ireland, it extends onto the limestone plateau of the Burren, occurring in the east Burren fens where drainage is impeded (D'Arcy & Hayward 1997).

1.3.4.3 *Plant communities*

In Europe, *C. dissectum* is a defining species in the Cirsio-Molinietum Siss. et De Vries 1942. This association has been recorded in Britain (Wheeler 1980) and in Ireland (White & Doyle 1982). In Ireland, *C. dissectum* has a wide synecology (Ó Críodáin & Doyle 1997). White & Doyle (1982) describe it as a characteristic species of the Junco Conglomerati – Molinion Westhoff 1968 and state that most of the wet grasslands in western Ireland belong to this alliance.

Braun-Blanquet & Tüxen (1952) defined a Cirsio dissecti – Schoenetum nigricantis association that was unique to Ireland, the characteristic species being *Schoenus nigricans,*

Cirsium dissectum, Anagallis tenella and *Hydrocotyle vulgaris.* White & Doyle (1982) describe the association as widespread throughout Ireland and Ivimey-Cook & Proctor (1966) found it to be the most widespread and characteristic fen type in the Burren. Ó Críodáin & Doyle (1997) do not support the Cirsio dissecti – Schoenetum nigricantis association, as defined by Braun-Blanquet & Tüxen (1952) and place this community in Ireland, within the Schoenetum nigricantis Allorge 1922. They define a new sub-association, the Cirsietosum dissecti, that comprises vegetation from the driest of habitats for *Schoenus nigricans.*

In Ireland, *Cirsium dissectum* is sometimes found on the edges of turloughs growing with *Carex panicea, Carex hostiana, Carex flacca, Molinia caerulea* and *Succisa pratensis* on nutrient-poor fens, often on skeletal limestone or with *Schoenus nigricans, Molinia caerulea, Achillea ptarmica* and *Parnassia palustris* in areas where a layer of fen peat is usually present (Goodwillie 2003); it is not, however, a characteristic turlough species.

Table 1.1 summarises the British National Vegetation Classification (NVC) communities in which *C. dissectum* occurs. It is a constant species within the M24 community and is described from a further six communities including wet heath, fen meadow, mires and tall herb fen (Rodwell 1991, 1995). Ross (1999) recorded *C. dissectum* from two dune slack sites but it is not recorded from any sand dune communities within the NVC.

Blackstock *et al.* (1998) surveyed 50 wet grassland sites in lowland Wales and examined edaphic and floristic characteristics within these. They suggested an additional sub-community called the Welsh nodum (M24x) within the M24 community to cover stands that had a poor representation of preferentials for the existing sub-community types. Three variants of M24x were described with *C. dissectum* occurring in two of these. Yeo *et al.* (1998) surveyed 114 remnant stands of neutral and acidic dry grasslands and wet

pastures in mid-Wales, *Cirsium dissectum* was most frequent in M24b (*Molinia caerulea – Cirsium dissectum* fen meadow, typical sub-community); M24c (*Juncus acutiflorus – Erica tetralix* subcommunity) and M24x but small populations were also recorded from a range of other community types.

Table 1.1 The National Vegetation Classification communities that contain *Cirsium dissectum* (derived from Rodwell 1991, 1995).

Communities and subcommunties	Constancy of *C. dissectum*	Constant species	Distribution	Habitat
M24 *Molinia caerulea* – *Cirsium dissectum* fen-meadow *Cirsio-Molinietum caeruleae* Sissingh & De Vries 1942 *emend* **M24a** *Eupatorium cannabinum* **M24b** Typical **M24c** *Juncus acutiflorus-Erica tetralix*	M24(IV) M24a(IV) M24b(IV) M24c(IV)	*Molinia caerulea, Potentilla erecta, Succisa pratensis, Cirsium dissectum, Lotus pedunculatus, Carex panicea.*	M24a is found in East Anglia, whilst M24b is found throughout lowland southern Britain, except for the SW. M24c is characteristic of the SW.	Moist to fairly dry peats and peaty mineral soils, circumneutral but only moderately mesotrophic, in the warmer lowlands of southern Britain. Typically managed with low intensity cattle grazing.
M16 *Erica tetralix-Sphagnum compactum* wet heath *Ericetum tetralicis* Schwickerath 1933 **M16a** Typical **M16b** *Succisa pratensis-Carex panicea*	M16(I) M16a(I) M16b(II)	*Erica tetralix* *Calluna vulgaris* *Molinia caerulea* *Sphagnum compactum*	M16a is found throughout southern Britain and as impoverished stands further north. M16b occurs in SW England.	Acid and oligotrophic mineral soils or shallow peats that generally have a surface pH of 3.5-4.5 and are at least seasonally waterlogged. M16b often occurs in areas with more base-rich soil.
M21 *Narthecium ossifragum-Sphagnum papillosum* valley mire *Narthecio-Sphagnetum euatlanticum* Duvigneaud 1949 **M21a** *Rhynchospora alba-Sphagnum auriculatum*	M21(I) M21a(II)	*Erica tetralix* *Molinia caerulea* *Eriophorum angustifolium* *Narthecium ossifragum* *Drosera rotundifolia* *Sphagnum papillosum* *Calluna vulgaris*	M21a is found in central southern England and the SW.	Permanently waterlogged, acid and oligotrophic peats, particularly characteristic of valley mires maintained by a locally high water table.
M22 *Juncus subnodulosus-Cirsium palustre* fen-meadow **M22b** *Briza media-Trifolium* spp. **M22d** *Iris pseudacorus*	M22(I) M22b(I) M22d(I)	*Juncus subnodulosus* *Calliergon cuspidatum* *Mentha aquatica* *Holcus lanatus* *Cirsium palustre* *Equisetum palustre* *Filipendula ulmaria* *Lotus pedunculatus*	M22b occurs throughout the southern British lowlands. M22d is especially well represented on topogenous mires in East Anglia.	A variety of moist, base-rich and moderately mesotrophic peats. pH range 6.5-7.5. Can be found either in, or around, well developed springs, flushes and mires.
M13 *Schoenus nigricans-Juncus subnodulosus* mire *Schoenetum nigricantis* Koch 1926 **M13a** *Festuca rubra-Juncus acutiflorus* **M13b** *Briza media-Pinguicula vulgaris* **M13c** *Caltha palustris-Galium uliginosum*	M13 (I) M13a (I) M13b (I) M13c (II)	*Carex panicea* *Juncus subnodulosus* *Schoenus nigricans* *Molinia caerulea* *Calliergon cuspidatum* *Succisa pratensis* *Potentilla erecta* *Campylium stellatum*	M13a found throughout lowland England and Wales but not recorded on the S coast and the SW. M13b and c are found in East Anglia and Anglesey	Peat or mineral soils, in and around lowland mires irrigated by base-rich, highly calcareous, and oligotrophic waters. Flushing waters typically have a pH between 6.5-8.
M29 *Hypericum elodes-Potamogeton polygonifolius* soakway *Hyperico-Potamogeton polygonifolii* (Allorge 1921) Braun-Blanquet & Tüxen 1952	M29 (I)	*Hypericum elodes* *Potamogeton polygonifolius* *Ranunculus flammula* *Sphagnum auriculatum* *Juncus bulbosus*	Confined to warm, oceanic parts of Britain, extends from west Surrey through the New Forest to the SW and north through Wales to Galloway.	Characteristic of shallow soakways and pools in peats and peaty mineral soils with fluctuating water levels, such as seepages and runnels around mires and in heathland pools. Water is typically clear, pH 4-5.5 and probably quite oligotrophic.
S24 *Phragmites australis-Peucedanum palustre* tall-herb fen *Peucedano-Phragmitetum australis* Wheeler 1978 *emend.* **S24f** *Schoenus nigricans*	S24(I) S24f(I)	*Phragmites australis* *Galium palustre* *Lysimachia vulgaris* *Peucedanum palustre* *Eupatorium cannabinum* *Lythrum salicaria* *Juncus subnodulosus* *Calliergon cuspidatum* *Calamagrostis canescens* *Filipendula ulmaria* *Mentha aquatica* *Cladium mariscus*	The most extensive tracts of this community occur in the middle reaches of the Yare, Bure, Ant and Thurne valleys in Broadland, east Norfolk.	Fen peats with a moderate to high summer water-table and some winter flooding with base-rich, calcareous and often oligotrophic waters. Water pH generally between 6.5-7.5.

1.3.5 Response to biotic factors

1.3.5.1 *Defoliation*

Cirsium dissectum is found in habitats that are either cut or grazed and sometimes burnt (Ross 1999; Jongejans 2004). In a growth room experiment, Ross (1999) discovered that *C. dissectum* was reasonably robust in its ability to withstand defoliation. Defoliated plants (with all of the leaves removed) showed a 35% decrease in root relative growth rate (RGR) and a 63% increase in shoot RGR, which allowed leaf biomass to be replaced in less than eight weeks. Replacement of the leaves depended on adequate nitrogen supply but was not particularly sensitive to low concentrations of phosphorus.

1.3.5.2 *Competition from other plants*

Cirsium dissectum is susceptible to being out-competed by plants that are able to increase biomass more rapidly, especially when nutrient levels are increased through the effects of fertiliser addition or natural succession. In an open greenhouse experiment, where *C. dissectum* plants were grown with and without a grass competitor (*Agrostis capillaris*), the below-ground presence of the grass reduced the average biomass of *C. dissectum* by a factor of 5.8 (Jongejans 2004).

1.3.5.3 *Animal feeders and pathogens*

C. dissectum is visited by a range of butterflies, bees and long-tongued flies and these are likely to be the agents of pollination (Kay & John 1994). The seed heads are predated by Tephritid flies, whose larvae eat the developing seeds (Spencer 1972; Redfern *et al.* 1995). The organisms recorded in or on *C. dissectum* in the literature are listed in **Table 1.2.**

Table 1.2 Species recorded in or on *Cirsium dissectum*. (Sources: 1. Spencer (1972), 2. White (1988), 3. Kay and John (1994), 4. Zwölfer and Harris (1984), 5. Borsje (2005), 6. Ellis and Ellis (1985)).

Species	Source	Ecological Notes
Insects		
Coleoptera		
Curculionidae		
Rhinocyllus conicus	4	Larvae and adults phytophagous, coastal.
Diptera		
Agromyzidae		
Phytomyza autumnalis	1	Larvae oligophagus.
Liriomyza strigata	1	Larvae polyphagous. Mining.
Tephritidae		
Chaetostomella cylindrica	2	Larvae feed and pupate within the capitulum
Terellia ruficauda	2	Larvae feed and pupate within the capitulum
Syrphidae		
Rhingia campestris	3	Larvae feed and pupate within the capitulum
Volucella bombylans	3	Flower visitor
Hymenoptera		
Apidae		
Bombus pascuorum	3	Flower visitor
Lepidoptera		
Nymphalidae		
Eurodryas aurinia	3	Flower visitor
Boloria selene	3	Flower visitor
Pieridae		
Pieris napi	3	Flower visitor
Papilio machaon britannicus	5	Flower visitor
Fungi		
Basidiomycota		
Uredinales		
Puccinia calcitrapae	6	Rust occasionally occurs on the leaves and stem
Ustilaginales		
Thecaphora trailii	6	Smut occasionally affects the flowers, fruits and seeds

1.3.6 Response to abiotic factors

1.3.6.1 *Light*

Ross (1999) investigated relative growth rate in *C. dissectum* at two light levels, 350 and 150 μmol m^{-2} s^{-1} photon flux density, in a growth room with a 16 h day at 22 °C and 15 °C night. Plants were grown in conical flasks containing Rorison nutrient solution. *C. dissectum* showed a 13% reduction in mean relative growth rate at the lower light level and Ross (1999) concluded that it was relatively tolerant of shade. Ellenberg (1988) gave *C. dissectum* a light indicator value of 7, a species of well-lit places, but which also occurs in partial shade, but Hill *et al.* (1999) gave *C. dissectum* within Britain a light indicator value of 8, a light-loving plant rarely found where relative illumination in summer is less than 40%.

1.3.6.2 *Water*

Ross (1999) investigated water uptake and water use efficiency using a gravimetric method; plants were grown in conical flasks containing Rorison nutrient solution and the use of water determined by weighing the plants and the conical flasks containing the solutions at regular intervals. *Helianthus annuus* was also grown so that the water use of this mesophytic species could be compared to *C. dissectum.* The experiment was carried out in a growth room with a 16 h day at 22 °C and 15 °C night with daytime light supplied at 350 μmol m^{-2} s^{-1} photon flux density. The mean relative water uptake was a factor of 1.5 times higher for *C. dissectum* compared to *H. annuus* but the mean water use efficiency (measured as dry matter / water used in transpiration) was almost identical. *C. dissectum* thus appears to have a relatively high water use but similar levels of water use efficiency compared to a mesophytic species.

1.3.6.3 *Nutrients*

Pegtel (1983) included *C. dissectum* in a glasshouse pot experiment, where plants were watered with nutrient solutions lacking in a range of nutrients. Solutions lacking phosphate and nitrate resulted in plants that showed very little increase in dry mass over time whilst the absence of sulphate caused little reduction in growth. The absence of potassium slowed down growth after two months and deficiency became visible as necrotic spots on the leaves. Absence of calcium and magnesium also slowed growth but to a lesser extent than potassium. Hayati & Proctor (1991) investigated plant responses to nutrients (Ca, Mg, N, P and K) added to pots of wet heath peat. This demonstrated that *C. dissectum* showed a strong positive response to lime but not to calcium chloride, and to added P but not to N or K.

Jongejans *et al.* (2006a) examined the effect of increased nutrients on *C. dissectum* in a common garden experiment. Individual rosettes of *C. dissectum* were surrounded by *Molinia caerulea* plants and the effect of nutrient enrichment (equivalent of 120 kg N ha^{-1} yr^{-1}) examined. The biomass of *M. caerulea* tripled in the nutrient-enriched plots and the increased competition caused a decrease in *C. dissectum* survival from 90% in un-enriched plots to 33% in enriched plots.

de Graaf *et al.* (1997) studied the effects of aluminium concentrations and Al:Ca ratios on the growth of *C. dissectum* in nutrient solution experiments. Al accumulation in the shoots was seen as Al concentrations in the nutrient solutions were increased and this correlated with a reduction in growth at high Al concentrations (200 – 500 µmol L^{-1}). Poor root development, yellowish leaves and reduced contents of Mg and P in the plants were observed; all indications of Al toxicity. These negative effects were partially counterbalanced when plants were grown in the same Al concentration but with increased Ca concentrations, resulting in lower Al:Ca ratios.

de Graaf *et al.* (1998) investigated ammonium toxicity in a hydroculture experiment using nutrient solutions that differed both in mineral nitrogen form and in ammonium concentration. It was found that plants performed better using nitrate as a nitrogen source than when ammonium was used, with increasing ammonium concentrations causing a reduction in growth. Lucassen *et al.* (2002) elaborated on the findings of de Graaf *et al.* (1998) by suggesting that ammonium as the sole nitrogen source only had a negative effect on *C. dissectum* when in combination with a low pH. Ammonium uptake at a rhizosphere pH of 4 resulted in decreased survival rate and biomass development. At higher pH or when nitrate was the sole nitrogen source, these effects were not seen. Similarly, Dorland *et al.* (2003) conducted glasshouse dose-response experiments examining the influence of ammonium on germination and survival: a significant negative correlation of both germination and survival with increasing ammonium addition was found at a pH of 4.3.

Franzaring *et al.* (2000) examined the response of *C. dissectum* to elevated ozone concentrations. After 28 days of ozone levels of 26.3 $\mu L\ L^{-1}$ (accumulated exposures over a threshold of 40 $nL\ L^{-1}$), a significant decrease in root mass and the root:shoot ratio was observed and after 113 days, a significant decrease in shoot mass was seen. These results were in marked contrast to *Molinia caerulea* which showed an increase in growth in response to elevated ozone.

1.3.7 Reproduction

1.3.7.1 *Seed production*

Jongejans *et al.* (2006b) recorded a range of 72 to 94 flowers per capitulum with 0.09 to 0.49 seeds per flower in un-predated capitula and 0 to 0.37 seeds per flower in predated capitula in three grasslands within the Netherlands. Flowering and seed production varied significantly over the five years that the grasslands were monitored.

1.3.7.2 *Germination*

Ross (unpublished data) investigated germination at 16, 23 and 31 °C for stratified (moist conditions at 5 °C for six weeks) and non-stratified (stored dry at 20 °C) seeds in growth conditions of a 16-h day at 43 μmol m^{-2} s^{-1} photon flux density and an 8-h night. Fifty seeds per treatment were germinated in Petri dishes lined with moist filter paper with each treatment having three replicates (**Figure 1.3**). The final percentage germination was higher and the time taken for half of the seeds to germinate (t50) was lower when the temperature was increased. In all cases, stratified seeds performed better than non-stratified seeds. Seeds were able to germinate in light and dark conditions.

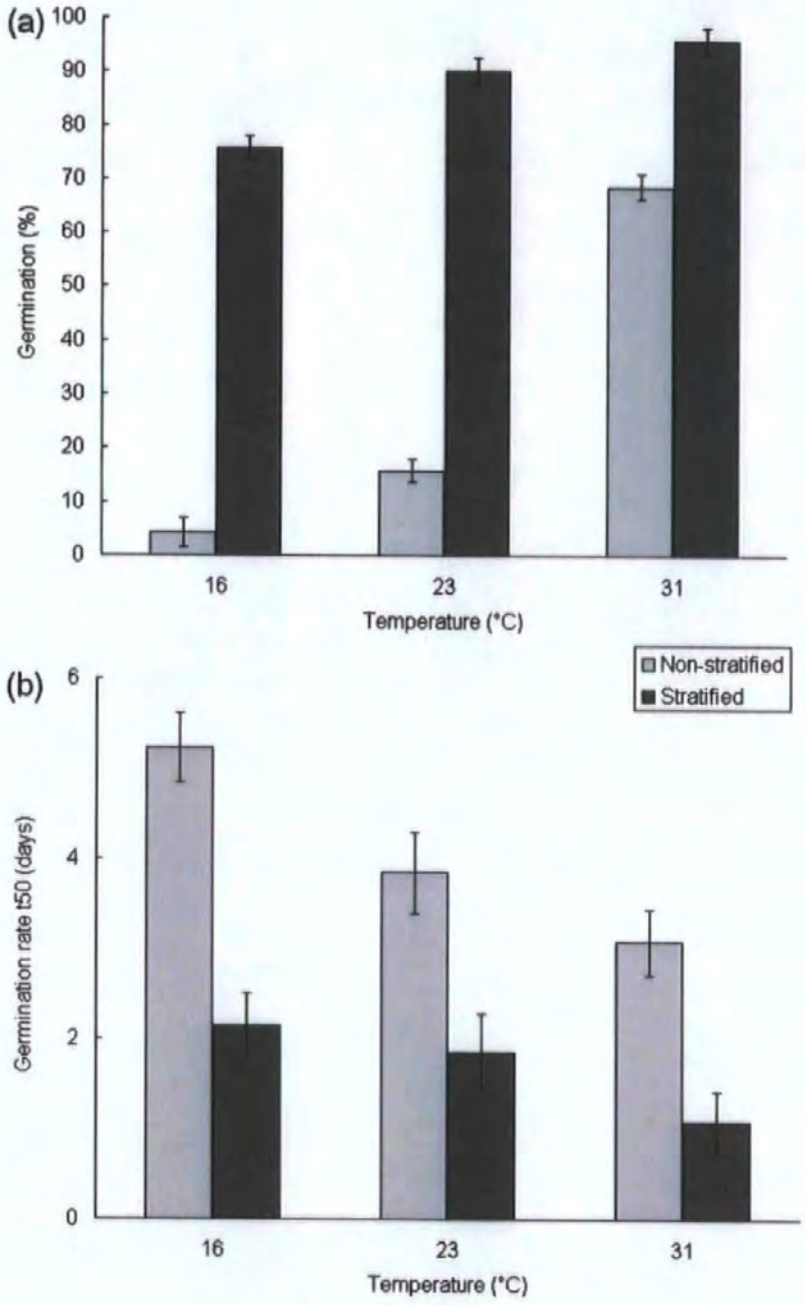


Figure 1.3 Germination of stratified and non-stratified seeds of *Cirsium dissectum*, (a) final percentage germination, (b) germination rate: time taken for 50% of the seeds to germinate (t50). (Graph adapted from J. Ross (unpublished data)).

1.3.7.3 *Seed dispersal*

Kay & John (1994) investigated seed dispersal at Kenfig, Wales and observed dispersal distances up to 10 m at wind speeds of approximately 2 m s^{-1} and up to 20 m at speeds of around 3 to 4 m s^{-1}. Simulated seed dispersal kernels determined by Soons *et al.* (2005) showed high probabilities of dispersal close to the parent plant, with dispersal probability dropping to almost zero at approximately 13 m. The wind speeds used in the model represented the average wind speed distribution during the dispersal season (June to October) in the interior of the Netherlands. Simulations estimated that 1 in 10,000 seeds would be dispersed over 3.4 km under stormy weather conditions, with an average horizontal wind speed of 22 m s^{-1}, at 10 m height (Soons 2006).

Soons & Heil (2002) investigated the effect of population size and site productivity on the ability of *C. dissectum* to colonise new areas in the Netherlands. To provide an estimate of productivity, three 20 x 20 cm vegetation plots were clipped at each site. These were dried and the mean dry mass determined. Colonization ability consisted of seed production, dispersal ability and germination. Smaller populations were found to have lower colonization capacity, as they produced fewer seeds per capitulum, had lower percentage germination (under glasshouse conditions) and a narrower range of seed dispersal distances. Sites with greater productivity had higher seed production and percentage germination was greater under glasshouse conditions. These factors should allow greater colonization capacity of nearby sites but this will only be possible if there are safe sites for seedling establishment. Seed dispersal ability decreased with greater productivity reducing the possibility of longer distance dispersal.

1.3.7.4 *Seedling establishment*

Isselstein *et al.* (2002) investigated seedling establishment by adding *C. dissectum* seeds to a Cirsio-Molinietum and a species-poor grassland under treatments including irrigation,

cutting of the surrounding vegetation and disturbance of the soil surface. Seedling establishment of *C. dissectum* was consistently higher on the Cirsio-Molinietum compared to the species-poor grassland but was still only 15% in the absence of any treatments. Disturbance of the soil and removal of the surrounding vegetation both significantly increased establishment levels. Jongejans *et al.* (2006b) and Soons *et al.* (2005) observed even lower seedling establishment after seed addition in a range of natural Cirsio-Molinietum grasslands within the Netherlands, although again establishment was higher in sites where topsoil had been removed as a restoration measure.

1.3.7.5 *Population dynamics*

Jongejans *et al.* (submitted) investigated the population dynamics of *C. dissectum* and the effect of site productivity using three approaches.

a) A demographic study was conducted over four years in three grasslands in the Netherlands.

b) The pattern of clonal spread and flowering was examined in a common garden experiment in which *C. dissectum* was grown with the competitor *Molinia caerulea* and the effect of nutrient addition investigated.

c) A site survey was carried out on five grasslands and two restoration areas were topsoil had been removed. These sites differed in productivity (estimated as the biomass of mown vegetation). In each site, biomass of *C. dissectum* was recorded and the rhizomes excavated to observe clonal spread.

The demographic study showed that sexual reproduction played a small role in the population dynamics of *C. dissectum* in established vegetation stands and that population growth was predominantly through clonal reproduction. Only three seedlings were found during the course of the study and no seedling survival was recorded. The very low number of seedlings meant that it was not possible to quantify seedling fate reliably in the

population dynamics model, so seedling growth and stasis was estimated at a conservatively high level. Even with this assumption, seedlings made up less than 0.1% of the projected stable stage distribution.

The common garden experiment showed that nutrient enrichment increased the biomass of *Molinia caerulea* but not *C. dissectum.* Instead, nutrient enrichment increased the proportion of *C. dissectum* rosettes that flowered and also increased rosette turnover. This meant that, even though rhizome lengths did not differ, *C. dissectum* in the nutrient-enriched plots was further away from the *Molinia caerulea* plants that were planted in the centre of the plot. Increased flowering and rosette turnover may act as escape strategies with increased flowering allowing seeds to establish in new areas, whilst clonal offspring move further away from high nutrient areas. However, if productivity is consistently high, ramets will not be able to escape and the seeds will not be able to establish, reducing the probability of survival.

The site survey largely supported the other findings; the greatest proportion of flowering rosettes was found in the most productive site but also in one of the topsoil removed restoration areas. The most productive site also had the greatest number of rhizomes per rosette. No seedlings were observed in the five grasslands but seedling establishment did occur in the two areas where the topsoil had been removed.

1.3.7.6 *Hybrids*

The hybrid *C. dissectum* × *C. palustre* = *C.* × *forsteri* (Sm.) Loudon is not infrequent where the parents occur and is the commonest hybrid thistle (Stace 1997; Preston *et al.* 2002). It is found throughout the range of *C. dissectum* in the British Isles and has been recorded from France and the Netherlands (Stace 1975). It has discontinuous spiny-winged, cottony pubescent stems and intermediate leaves and capitula. *C. dissectum* × *C. acaule* = *C.* × *woodwardii* was known from Pen Hill, Swindon, N. Wilts between

1848 and 1952 and recorded at South Lopham Fen, E. Norfolk, in 1953 (K.J. Walker, pers. comm.).

1.3.8 Genetics

A limited amount of previous research has examined genetic structure within *C. dissectum.* Kay & John (1994) examined levels of genetic diversity within and between populations of *C. dissectum,* predominantly in Wales, using allozyme markers. They found that eight loci in different enzyme systems proved reliable but only four of these were polymorphic. Twenty out of 22 Welsh populations were monomorphic and identical and a single population from Scotland was also monomorphic with the same genotype. Of the four English populations examined, only one showed variation at one locus. Levels of variability were higher in Ireland, where five out of seven populations showed either one or two polymorphic loci. The very low levels of polymorphism limited the conclusions that could be drawn from their results. They were unable to determine whether more than one genet was present within populations and could not calculate measures of genetic differentiation. They concluded that the amount of outcrossing and genet recruitment was very limited in *C. dissectum* and that Welsh populations represented a genetically depleted marginal group.

Smulders *et al.* (2000) used amplified fragment length polymorphism (AFLP) to investigate genetic diversity between source and reintroduced populations of *C. dissectum* in the Netherlands. Five source populations were sampled varying from a few kilometres to a maximum of 200 km from the reintroduction site. Two thousand seeds from each source population were introduced into experimental plots at the restoration site and these were genotyped using AFLP the following year. Source populations showed small but significant genetic differences between each other ($\Phi_{ST} = 0.108$). The first generation of reintroduced plants showed less genetic variation than their source populations and were also genetically differentiated from them. Assignment tests showed however, that

reintroduced populations still resembled their source populations more than any other population.

In contrast to the results obtained by Kay & John (1994), Smulders *et al.* (2000) recorded much greater genetic variation within *C. dissectum* populations and concluded that it was an outcrossing species in the Netherlands. Out of the 25 to 35 plants sampled for each population, none showed the same banding pattern. This suggests that each plant was a different genet (M. Smulders pers. comm.).

1.3.9 Conservation

Cirsium dissectum has been lost from many sites in the British Isles due to drainage and succession (Fojt & Harding 1995; Preston *et al.* 2002). In Germany and the Netherlands, drainage, acidification, atmospheric nitrogen deposition, fertilizer use and succession have caused large losses in *C. dissectum* and remaining sites are often small and fragmented (Jansen *et al.* 1996; Buck-Sorlin 1993; Buck-Sorlin & Weeda 2000; Jongejans 2004; van den Berg *et al.* 2005). Soons *et al.* (2005) related seed dispersal ability to the availability of suitable habitat within an area of the Netherlands to investigate habitat connectivity. The remaining grasslands containing *C. dissectum* were found to be practically isolated from each other in terms of seed dispersal with the regional survival of the species being completely dependent on a few large populations in nature reserves. Due to these factors, *C. dissectum* is now on the Dutch Red List of Endangered Species (Rossenaar & Groen 2003; Jongejans *et al* 2006a).

Research has been conducted in the Netherlands (Berendse *et al.* 1992; Jansen & Roelofs 1996; Jansen *et al.* 1996; Beltman *et al.* 2001) and Britain (Tallowin & Smith 2001) into the best methods for restoring Cirsio-Molinietum grasslands. Topsoil removal to decrease soil fertility in areas with a suitable hydrological regime has proved to be the most successful approach. The UK Biodiversity Action Plan lists purple moor grass and rush pasture as a priority habitat with plans to re-create this habitat on land adjacent to or

nearby existing sites (HMSO 1995) and van Soest (2001) has developed a methodology for the identification of suitable restoration sites in south-west England.

1.4 Research rationale

1.4.1 Ecology

Previous studies on *C. dissectum* have covered many aspects of the species' ecology but the preceding review highlights two areas were further research is required: reproductive biology and communities. Some observations have been made on the reproductive biology of *C. dissectum*, especially germination and seedling establishment, but the basic reproductive biology of this species within the British Isles has not been described and the pattern of clonal growth is largely unknown. *C. dissectum* has been described from a range of wet grasslands, heaths and fens throughout the British Isles. It can also occur on dune slacks but the plant species it is associated with in this habitat have not been described. The review also highlights the importance of soil nutrient levels and site productivity in the population dynamics of *C. dissectum*, but previous studies have not described these characteristics for sites throughout the British Isles.

1.4.2 Genetics

Two studies have examined the genetics of *C. dissectum* and reported contrasting results using different genetic markers (Kay & John 1994; Smulders *et al.* 2000). Allozymes and AFLP are at opposite ends of the spectrum in the level of polymorphism they detect (Lowe *et al.* 2004). For *C. dissectum* in the British Isles, the very low level of polymorphism detected using allozymes limited the conclusions that could be drawn from those data. Microsatellite genetic markers detect higher levels of polymorphism than allozymes and,

unlike AFLP, the data are codominant. Beaumont & Bruford (1999) have suggested that microsatellites are ideal for use in conservation biology and population genetics. Microsatellite genetic markers have been developed for *C. acaule* by Jump *et al.* (2002) and their utility tested in other *Cirsium* species (although this did not include *C. dissectum*). These markers may be appropriate for use in *C. dissectum.*

The previous review highlights that, in established vegetation stands, clonal reproduction is the dominant form of population increase. Increasing nutrient levels increases the proportion of rosettes that flower but seedling establishment is very low and seedlings were only found in areas where disturbance produced safe sites for establishment. Seedling establishment may only be seen at early successional stages. This has implications for genetic diversity, as the absence of sexual reproduction may lead to reductions in genetic diversity over time. Levels of genetic diversity may therefore depend on habitat, with nutrient levels and productivity being particularly important. The habitat available to *C. dissectum* has also declined in size and increased in isolation, factors that are known to lead to reductions in genetic diversity and increases in inbreeding. Both these factors can lead to a reduction in fitness in plant populations. Examining the relationships between habitat quality, population size, genetic diversity and fitness in *C. dissectum* can be used to investigate some of these suggestions and provide information on the best way to manage sites.

The loss and fragmentation of *C. dissectum* habitat is likely to have led to greater isolation between populations and this will influence levels of genetic differentiation. An understanding of how genetic variation is partitioned within and between populations can help to identify populations that it is important to conserve. Furthermore, as plans are in place to re-create habitat areas including *C. dissectum* in Britain and the Netherlands, an understanding of genetic differentiation is important in order to decide the best populations

to use for restoration projects. This can be supplemented with an investigation into the effect of inbreeding and outbreeding by carrying out experimental crosses.

Finally, the use of neutral genetic markers such as microsatellites has been criticised, as they may not relate to selectively important variation (Booy *et al.* 2000; Ouborg *et al.* 2006). This can be investigated by comparing variation in morphological traits with microsatellite genetic markers.

1.4.3 Aims and objectives

The overall aim of this research is to describe the ecology and genetics of *C. dissectum* within the British Isles and to consider the conservation implications of the results. This will be achieved through the following aims and objectives.

1. **Explore the ecology of *C. dissectum* within the British Isles by examining site characteristics, plant communities and reproductive biology.**
    a) Describe site characteristics in a range of populations by recording soil variables, vegetation structure and site management.
    b) Describe the range of plant communities within which *C. dissectum* is found in Britain including wet grasslands, heaths, fens and dune slacks.
    c) Examine the reproductive biology of *C. dissectum* by describing plant phenology, morphology of sexual and vegetative reproductive organs, seed production, germination, dispersal, animal feeders and pollinators and hybridization.
    d) Describe the number and distribution of clones in different populations using microsatellite genetic markers.
2. **Examine genetic diversity within populations and relate this to population size, plant fitness and habitat quality.**
    a) Examine levels of genetic diversity and inbreeding within *C. dissectum* populations.

b) Investigate the interactions between genetic and genotypic diversity, population size, habitat quality and plant fitness.

c) Consider the implications of the results for the management of *C. dissectum* populations.

3. **Describe genetic differentiation in *C. dissectum* and investigate the effect of inbreeding and outbreeding.**

a) Examine how genetic variation is partitioned within *C. dissectum*.

b) Describe genetic differentiation between populations and test whether genetic variation can be explained through isolation by distance.

c) Investigate whether plants that are genetically different, as measured using microsatellite markers, have morphological differences when plants are grown in standard conditions.

d) Conduct intra- and inter-population crosses to test whether *C. dissectum* is self-compatible and investigate the effects of inbreeding and outbreeding on seed production and germination.

e) Consider the implications of the results for the conservation of populations and the source of material for habitat re-creation projects.

1.4.4 Thesis outline

Chapter 2 describes all of the general methods used throughout the rest of the thesis, including the populations surveyed and site characteristics, community analysis, measuring population size, reproduction and morphological variation, crossing within and between populations and analysing genetic diversity using microsatellite genetic markers. Chapter 3 provides an overview of the site characteristics for the populations of *C. dissectum* surveyed; reproductive biology and plant communities within Britain are described. Chapter 4 describes genetic diversity within populations of *C. dissectum* and examines the interactions between population size, genetic diversity, plant fitness and the habitat

variables described in Chapter 3. Chapter 5 continues the examination of the genetics of *C. dissectum* by covering how genetic diversity is partitioned within and between populations and examining levels of genetic differentiation. Differences observed using microsatellite markers are then related to morphological differences between plants grown in standard common garden conditions. The implications of genetic differences are considered by carrying out crosses within and between populations to examine the potential effects of inbreeding and outbreeding. Chapter 6 is the general discussion and summarises the findings of the other chapters, conservation implications and further work are then discussed.

Chapter 2

2 Methods

2.1 Selection of sites containing *Cirsium dissectum*

Sites were chosen that were in the main distribution area for *C. dissectum* within the British Isles, with the extremities of the distribution avoided, in order to prevent edge of range effects (Jump 2002, Gaston 2003). Potential site information was obtained from the Botanical Society of the British Isles vice-county recorders, Kay & John (1994), Ross (1999), lists of SAC sites and the Culm grassland database maintained by the Devon Wildlife Trust. Using the information provided from these sources, a short-list was drawn up, based on the need to cover the whole of the distribution area and to have approximately equal number of populations in both Britain and Ireland. A range of different distances between sites was required to ensure that isolation by distance could be investigated. The sites also needed to cover a full range of population sizes and capture the variation in habitat types in which *C. dissectum* was found. Permission to visit sites and relevant permits as required were obtained before collecting data, seeds, plant parts or soil samples.

Table 2.1 lists the sites, their position is shown in **Figure 2.1**, and Appendix A provides site descriptions. Data were collected in four field campaigns:

a) 28/07/02 – 18/08/02. Plant community data were collected from 11 sites in Britain using quadrats to identify the plant species associated with *C. dissectum.*

b) 11/05/03 – 30/06/03. Leaf material was collected from 22 sites for use in the genetic analysis. A small number of seed heads were collected from three Irish populations.

c) 30/06/03 – 03/08/03. Thirty seed heads were collected from each of 12 sites in Britain; these seeds were raised in standard conditions and used for crossing experiments and morphological measurements.

d) 03/07/04 – 22/07/04. The 22 sites, where leaves were collected for genetic analysis, were revisited, population area measured, number of flowers counted and quadrats were used to measure vegetation height, bare soil, proportion of flowering rosettes and plant density. Soil was collected for soil analysis. Thirty seed heads per population were collected to determine seed production, germination, survival and morphological variation. In addition to the field campaigns described above, five sites were visited at various seasons throughout the year from 2002 – 2006 to make general observations (**Table 2.1**).

Table 2.1 Site locations and codes for *Cirisum dissectum* populations surveyed and summary of the data collected. (C: community data; P: population area and flowering; S: vegetation structure and soil analysis; R: reproductive data; M4: morphological variation 2004; M3: morphological variation 2003; X: crossing; G: genetic analysis; O: observations throughout the year).

Code	Site location	Grid ref.	Data collected
EAC	Aylesbeare Common, England	SY052910	C P S R M4 M3 X G O
EBB	Braunton Burrows, England	SS459351	C P S R M3 X G O
EBC	Baddesley Common, England	SU390217	C
EKS	Knowstone Moor, England	SS844215	C P S R M4 M3 X G O
EMM	Mambury Moor, England	SS385171	C P S R M4 M3 X G
EMO	Marlpitt Oak, England	SU285002	C P S R G
EMS	Meshaw Moor, England	SS758187	C P S R M4 M3 X G O
ERW	Rans Wood, England	SU362031	C P S R G
EWF	Wicken Fen, England	TL562705	C P S R M4 X G
IAR	Lough Corrib, Ireland	M170434	P S R M4 X G
IBL	Bleach Lough, Ireland	R441557	P S R M4 X G
IDL	Doagh Lough, Ireland	H079526	P S R M4 G
IGC	Giants Causeway, Ireland	C944445	P S R M4 G
ILB	Lough Bunny, Ireland	R382979	P S R M4 X G
ILG	Lough Gealain, Ireland	R316954	P S R M4 G
ILL	Lough Lattone, Ireland	H009470	P S R G
ILT	Lough Talt, Ireland	G397161	P S R G
IMA	Marble Arch, Ireland	H117352	P S R G
IME	Meencargagh, Ireland	H289784	P S R M4 G
WCG	Cefn Cribwr, Wales	SS866835	P S R M4 M3 X G
WDB	Drostre Bank, Wales	SO096318	C P S R M4 M3 X G
WKF	Kenfig, Wales	SS784816	C P S R M3 X G O
WWM	Welsh Moor, Wales	SS517932	P S R M3 X G

Figure 2.1 Distribution of *Cirsium dissectum* and codes for the sites surveyed. (Purple: distribution 1987 to 1999; pink: distribution pre-1970 to 1986. Map from Preston *et al.* (2002)).

2.2 Plant community data

During July and August 2002, 11 sites within England and Wales were surveyed to determine the types of communities within which *C. dissectum* was found. Sites were chosen that appeared to represent the greatest amount of variation in community type. Within each site, 10 2 x 2 m quadrats were positioned and within each quadrat, all species were identified and their abundance estimated using the Domin scale (**Table 2.2**).

Table 2.2 Domin values (*sensu* Rodwell 1991)

Domin value	Estimated plant cover (%)
1	< 4 with few individuals
2	< 4 with several individuals
3	< 4 with many individuals
4	4 – 10
5	11 – 25
6	26 – 33
7	34 – 50
8	51 – 75
9	76 – 90
10	91 – 100

2.3 Population size

For the purposes of this study, a population was considered to be a continuous group of plants separated from other groups by a distance of at least 200 m. Soons *et al.* (2005) and Kay & John (1994) showed that most dispersal occurred close to the parent plant, up to 20 m, so plants separated by 200 m are likely to be reproductively isolated, except for rare long-distance dispersal events. Due to the isolated nature of *C. dissectum* habitat, large distances often separated sites, but some locations (Aylesbeare Common (EAC), Braunton Burrows (EBB), Kenfig (WKF)) did have more than one area containing *C. dissectum*, in this case the largest population was sampled.

Population size is often estimated by counting the number of flowering individuals within a population (Kéry *et al.* 2000; Vergeer *et al.* 2003; Matthies *et al.* 2004), as this is

assumed to provide an estimate of effective population size (Frankham *et al.* 2002). The number of flowering rosettes was therefore counted in July 2004. Site visits in previous years had shown considerable variation in the proportion of rosettes flowering within different populations, so the census population size (total number of rosettes) was also estimated as the product of population area and density. It should be noted however that it was not possible to distinguish between individual clones in the field, so this was an estimate of ramet rather than genet number.

Population area was measured using a global positioning system (Garmin eTrex, UK) to provide the latitude, longitude and altitude of plants on the periphery of each population. Software developed by A. Read (Spirent Communications plc, source code available on request) was then used to connect the recorded positions together and measure the area covered (**Figure 2.2**). To estimate plant density, 30 1 x 1 m quadrats were positioned throughout the site. This was achieved by walking a transect along the longest length of the population and throwing a marker at various intervals, the quadrat was placed were the marker landed. In each quadrat, the number of flowering and vegetative rosettes was counted and the mean number of all rosettes per m^2 was used as the measure of density. The proportion of flowering to vegetative rosettes was also calculated.

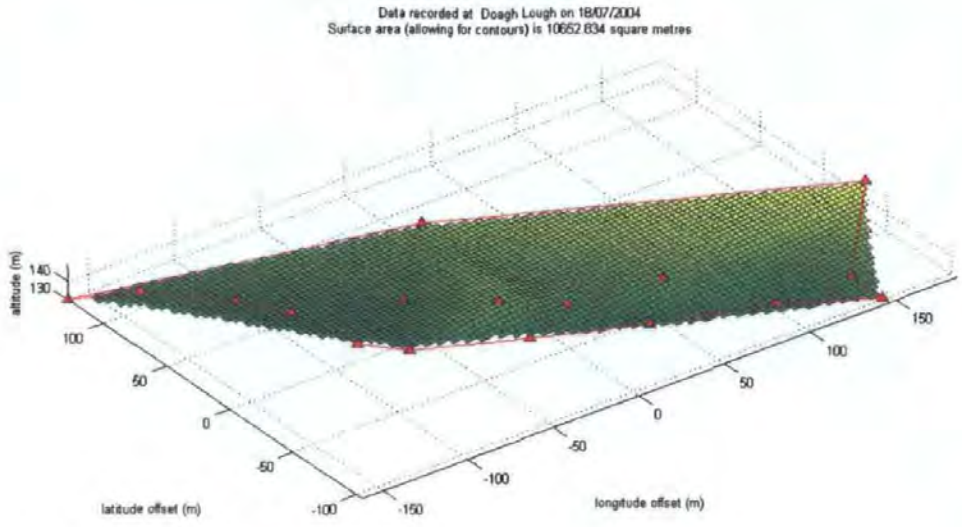


Figure 2.2 Example of population area determined using software developed by A. Read (Spirent Communications plc). (Red triangles indicate each of the GPS readings taken at the site. The software connected the GPS readings taken at the periphery of the population and measured the area inside the red line. The colour on the map indicates the altitude with yellow being higher and dark green lower).

2.4 Vegetation structure, soil analysis and site management

The mean vegetation height and percentage cover of bare soil were used to provide measures of the structure of the vegetation. These were measured in the same 30 1 x 1 m quadrats in which the number of rosettes was counted. Where possible site management was ascertained by talking to site owners and managers.

For each site, five topsoil cores were sampled (depth 14 cm, diameter 3 cm) with an auger. The top and bottom halves of the soil core were separated and the top and bottom samples pooled for the site. The top (0 to 7 cm) and bottom (7 to 14 cm) of the soil core were separated to investigate differences in nutrient profiling. The 7 to 14 cm samples provided soil measures in the main rooting zone of *C. dissectum*. The concentrations of the

following were measured in the soil samples: nitrogen, phosphorus, potassium, calcium, pH and organic matter. Due to the large number of sites that needed to be visited in a short time, it was not possible to use fresh samples for soil analysis so all samples were air-dried. This meant that some potential analyses could not be undertaken, for example, it would have been valuable to measure soil ammonium, as this has toxic effects at low pH (de Graaf *et al.* 1998; Lucassen *et al.* 2002; Dorland *et al.* 2003). Similarly, it would have been useful to measure soil water content but comparisons between sites measured over a three-week period would not have been valid. The methods used for soil analysis followed those adopted by Ross (1999) to enable comparisons to be made between the results.

2.4.1 Total Kjeldahl nitrogen

Total Kjeldahl nitrogen is based on the wet oxidation of soil organic matter using sulphuric acid and a digestion catalyst which converts organic nitrogen to ammonium. The amount of ammonium is then determined using titration. The procedure does not digest nitrogen from heterocyclic compounds, oxidised forms such as nitrate and nitrite, or ammonium from within mineral lattice structures (Goodwin 1995; Ross 1999). 1 g of air-dried soil, 2 tablets of Kjeltass TCT digestion catalyst (composed of K_2SO_4 and HgO in the ratio of 20:1) and 13 mL of concentrated sulphuric acid were placed into a digestion tube. This was left in a digester for 1 hour, during which time a lime green colour developed. After dilution with 50 mL of distilled water, the tube was placed into a Kjeltec System 1002 (Tecator, Sweden) distilling unit and 50 mL distillate collected into a flask containing 50 mL of boric acid indicator. (The boric acid indictor was made by dissolving 20g of H_3BO_3 in water, adding 15 mL of BDH '4.5' indicator and diluting to 1L (Allen 1989)). The resulting solution of the distillate and boric acid indicator was titrated against 0.05 M sulphuric acid.

2.4.2 Extractable phosphorus

Extractable phosphorus was determined using Olsen's method (Allen *et al.* 1989). 5 g of air-dried soil was shaken with 100 mL 0.5 M sodium bicarbonate (Olsen's solution) at a pH of 8.5 for 30 minutes and then filtered. The resulting solutions had variable colorations due to different amounts of dissolved organic matter, with the peaty soils resulting in dark brown extracts. To remove the coloration, 25 mL of filtrate were shaken for 30 minutes with 0.5 g activated charcoal (Sigma-Aldrich, Poole, UK). The standard phosphorus solutions at 2.5, 5.0, 7.5 and 10.0 M, used to produce the calibration graph, were subjected to the same procedure to account for any additional phosphorus extracted by the charcoal. To 5 mL of the transparent filtrate were added 1 mL 1.5 M sulphuric acid, 20 mL 0.15% w/v ammonium molybdade and 5 mL ascorbic acid. This will neutralise the sample and allow a blue coloration to develop. The procedure was repeated with each of the standard phosphorus solutions and the sodium bicarbonate blank. The intensity of the blue coloration indicated the concentration of phosphorus developed, and this was measured after 20 minutes, using a spectrophotometer set at 880 nm.

2.4.3 Extractable potassium

Extractable potassium was determined by shaking 10 g of air-dried soil with 50 mL of 1 M ammonium nitrate for 30 minutes. After filtering, the extract was aspirated into an air-acetylene flame and the atomic emission determined using an atomic absorption spectrophotometer (Varian Spectr AA 50, Varian, UK).

2.4.4 Exchangeable calcium

5 g of air-dried soil were shaken with 100 mL of 1 M ammonium acetate at pH 7.0 for two hours and then filtered. 1 mL of filtrate was added to 1 mL lanthanum chloride, to prevent interference from other elements such as phosphate, aluminium and silicon (Allen *et al.*

1989). The solution was then made up to 100 mL and four calcium standards made up in the same way. The calcium content of the standards and then the soil sample was determined by air-acetylene flame absorption, using an atomic absorption spectrophotometer.

2.4.5 Soil pH

Soil pH was determined electrometrically after mixing 10 g of air-dried soil with 25 mL of distilled water, stirred for one minute, left to stand for 15 minutes and then a reading taken.

2.4.6 Organic matter

Organic matter was measured using loss on ignition. 2 g of air-dried soil were placed in a Muffle furnace (Carbolite, Sheffield, UK) at 800 °C for two hours. The mass before and after ignition was determined to calculate the amount of organic matter that was burnt off.

2.5 Reproduction

Thirty seed heads were collected from each of 22 populations in July 2004, except in very small populations, where approximately 10% of the seed heads available were collected to avoid over-sampling. Seed heads had to be ripe but not yet dehiscent. Each seed head was placed individually into a paper bag and allowed to air-dry. Flower heads were dissected by hand and the seeds removed. Each seed was pressed gently with a pair of forceps to ascertain if it contained an embryo and only those that felt hard were subsequently counted. Levels of predation were recorded and invertebrates that were found inside the seed head, and were preserved inside the bags, were identified. The mass of hard seeds per capitulum was determined by weighing all of the hard seeds contained in a single

capitulum together using a 5-decimel-place balance. This mass was then divided by the number of hard seeds in that capitulum to provide an average mass value per seed for each seedhead.

Germination was carried out using un-stratified, hard seeds. Seeds were placed in Petri dishes on three pieces of Whatman No. 1 filter paper that had previously been soaked in a solution of fungicide, made up as per the manufacturer's instructions (Bio Cheshunt Compound, Home and Garden Ltd, Waltham, UK). Each Petri dish contained the seeds from one seed head. Petri dishes were arranged randomly on a heated bench set at 30 °C in a glasshouse and water added to each Petri dish once or twice daily (depending on weather conditions) using a pipette. A seed was considered to have germinated as soon as the radicle was visible and germination was monitored on a daily basis until no new germination was observed for two weeks.

Germinated seeds were removed and placed singly in a 12 x 7 cell module tray filled with seed compost (Coir based tray and modular compost, Goldengrow, UK). Module trays were arranged randomly on a bench in an unheated glasshouse. When the seedlings had reached a suitable size for potting on, survival was recorded and all of the seedlings potted on individually into 7 x 7 cm pots. These were arranged randomly into trays and placed in an unheated polytunnel. Plants were subsequently potted into 15 cm diameter pots and placed in a net-tunnel.

2.6 Morphological variation

Morphological traits were measured on plants collected over two years. In 2003, seeds were collected from 12 populations in England and Wales and grown in the same conditions as described above. Fourteen morphological characters (**Table 2.3**) were

measured on one-year old vegetative rosettes, which, due to plant mortality, came from nine populations; 664 plants were measured in total with approximately equal numbers from each population (**Table 2.1**). These data (2003) were collected by S. Mayers (Liverpool John Moores University) and used for his undergraduate dissertation. Data collection was repeated in the same way the following year (by the author) using the seeds collected in 2004 from all 22 populations. Low germination and high seedling mortality in 2004 reduced the number of populations with sufficient plants to 14; 395 plants were measured with approximately equal numbers from each population.

The number and size of leaves are often correlated with the total biomass of the plant (Jongejans 2004). This was investigated using 217 one-year-old plants from five populations. For each plant the number of leaves and the length and width of the longest leaf was measured. The plants were then removed from the soil, cleaned and separated into above and below ground parts. All parts of the plants were air-dried for three days at 72 °C and the mass of the above and below ground parts was then determined. Pearson correlation coefficients were examined between above, below and total biomass and the following measures: number of leaves; leaf length; leaf width; leaf length x leaf width; number of leaves x leaf length; number of leaves x leaf width and number of leaves x leaf length x leaf width. Variables were log-transformed to improve normality. Leaf length x leaf width was found to have the highest correlation with total plant biomass (Pearson correlation coefficient = 0.795, *P*-value = < 0.0001. Leaf length x leaf width therefore provided a surrogate measure of total biomass that could be measured without destroying the plant.

Table 2.3 Characters measured on one-year old plants grown from seed collected in 2003 and 2004.

Character	Description
Number of leaves	If more than one ramet was present, characters were measured on the mother plant.
Leaf length (mm)	All leaf characters were measured on the longest leaf.
Leaf width (mm)	Measured at the widest point on the longest leaf.
Petiole length (mm)	
Number of veins	The number of main veins was counted on the right hand side of the midrib.
Spine length (mm)	The length of the longest spine.
Angle at base of leaf (degrees)	Where the petiole joins the midrib, the angle was measured from the leaf lamina to the midrib on the right hand side of the leaf. This provided a measure of the shape of the base of the leaf.
Angle at apex of leaf (degrees)	At the tip of the leaf, the angle between the midrib and the leaf lamina on the right hand side of the leaf was measured. This provided a measure of the shape of the tip of the leaf.
Undulation (mm)	The maximum amplitude perpendicular to the leaf lamina was measured.
Number of ramets	The number of clonal offspring in addition to the mother plant.
Leaf length x width (mm^2)	This variable is correlated with total plant biomass.
Leaf width widest: leaf length ratio	This provides a measure of the shape of the leaf, some leaves were more lanceolate and others more elliptical.
Petiole: leaf length ratio	Some plants appeared to have very short petioles whilst others were longer relative to the length of the leaf.
Pinnatifid ratio	The leaf width at the widest point of a lobe and the narrowest point was measured: leaf width narrow / leaf width widest = pinnatifid ratio. This provides a measure of leaf dissection.

2.7 Crossing within and between populations to investigate the effects of inbreeding and outbreeding.

Crosses were performed on 119 two-year old plants from 13 populations. These were from the seed collected in 2003 and grown in standard conditions in the glasshouse and net-tunnel. The categories of crosses were:

1. Selfed within a capitula (19 plants).
2. Within population (14 crosses).
3. Populations up to 30 km apart (9 crosses).
4. Populations 35 – 100 km apart (15 crosses).
5. Populations 110 – 450 km apart (12 crosses).

Flowering started from the beginning of June and carried on throughout July, so the categories chosen and the crosses performed depended on which plants flowered and when capitula were at a suitable stage for crossing.

When bud formation was developed, but before any florets had opened, the entire plant was enclosed within a muslin bag with a fine mesh. When the first florets opened, the plant was paired with another plant at a similar stage of development. Once the florets had opened, pollen was transferred using a paintbrush between the two parents or within the florets in the case of selfed capitula. Crosses were carried out reciprocally, so pollen was transferred between both parents. A disadvantage of this technique is that selfing is also possible in addition to cross-pollination. This method was adopted, however, as emasculation was considered impractical due to floret development occurring over a number of weeks and the anthers being connate and forming a tube around the style. Each cross was performed every day until the capitula had finished flowering. Once the seed head was ripe but not dehiscent, it was collected and allowed to air-dry; seeds were then dissected and the number of hard seeds counted. Seeds were germinated in 5% water agar in an incubator with a 12 h day at 23°C. The number of hard seeds and their germination were determined by A. Whotton (University of Plymouth) and the data used for his undergraduate dissertation. The data collected by the undergraduate students associated with this project were extensively checked, reworked and analysed by the author and are included as an integrated part of this thesis.

2.8 Genetic Analysis

Microsatellite genetic markers were used to investigate the genetics of *C. dissectum*. Microsatellites consist of short sections of repeated units of nucleotides with typically one

to six base pairs being repeated e.g. $(AT)_n$, $(CAA)_n$, $(GCTC)_n$. These microsatellites are scattered throughout the genome of most, if not all organisms, with eukaryotes typically containing hundreds or thousands of microsatellite loci. The great majority of microsatellites are in non-coding regions and are thus assumed to be selectively neutral (Beebee & Rowe 2004).

Microsatellites have high mutation rates, so there is generally variability between individuals in the number of nucleotide repeats, resulting in high levels of polymorphism. In addition, microsatellite data is co-dominant meaning that homozygotes and heterozygotes can be scored. High polymorphism and codominance mean that microsatellites are ideal for use in population genetics and conservation biology (Hancock 1999; Beebee & Rowe 2004).

A disadvantage of microsatellites is that the intial identification of the microsatellite loci is time-consuming and expensive, as it requires cloning and sequencing. Microsatellite loci also tend to be species specific, especially in plants, although cross-species amplification is sometimes possible (Lowe *et al.* 2004).

Microsatellite loci were not available for *C. dissectum*, but nine loci (from the nuclear genome) had been developed for *C. acaule* and these loci had also worked successfully in *C. arvense* and *C. heterophyllum* (Jump *et al.* 2002). These loci were therefore tested for use in *C. dissectum.*

2.8.1 Sampling procedure

In order to determine the best time of year to collect leaf samples, *C. dissectum* leaves from glasshouse grown plants were tested to compare the quality of DNA extracted from: young leaves; older leaves with the spines removed and older leaves with the spines left on. A phenol chloroform extraction based on Doyle and Doyle (1987) was performed and the resulting DNA compared for purity and concentration using a spectrophotometer set at 260 and 280 nm.

$$DNA Concentration = \frac{50 \times A_{260} \times V}{1000 \times n}$$

Equation 2.1 Where, A_{260} = absorbance at 260 nm, V = initial number of μL of TE buffer placed in cuvette, n = total number of μL of DNA sample placed in cuvette.

$$DNA purity = \frac{A_{260}}{A_{280}}$$

Equation 2.2 Where, A_{260} = absorbance at 260 nm and A_{280} = absorbance at 280 nm.

A microsatellite primer pair (Caca1) was then used to amplify the resulting DNA using the polymerase chain reaction (PCR) in a PTC-100 thermocycler (MJ Research) and the resulting DNA run on a 1% agarose gel using electrophoresis (see section 2.8.3). Young leaves performed better providing good quality DNA that resulted in sharp, bright bands on the agarose gel. It was therefore decided to sample leaves from field sites in May and June when new leaves were being produced.

A full sampling of leaf material was carried out in May and June 2003 from 22 populations, with 35 leaves taken from each population. The principal aim of the sampling strategy was to obtain enough individual plants to determine genetic diversity and differentiation within and among populations. Thirty-five plants were therefore sampled from each population with each plant 10 m from the next, in an attempt to avoid sampling the same clone numerous times and reducing the effective sample size. However, determining clonal structure within *C. dissectum* was a secondary aim, so a systematic sampling strategy was used and the position of all sampled plants was recorded, so that if clones were found, their positions could be plotted.

A transect was set up along the longest axis of the population and plants sampled every 10 m. If 35 plants were not collected by the end of the transect, another transect was set up 10 m away and the process repeated. For very small populations, this procedure was not possible, as insufficient plants were collected using the 10 m distance between samples. Thus for three populations, Braunton Burrows (EBB), Meencargagh (IME) and

Welsh Moor (WWM), plants were sampled every 5 m along each transect, whilst the 10 m distance between transects was maintained, and for the smallest population, Wicken Fen (EWF), plants were sampled in a 2.5 x 2.5 m grid. In addition, in population EWF, the size and position of every patch of *C. dissectum* was measured.

Collecting 35 leaf samples from every population meant that smaller populations were sampled at a greater intensity than larger populations. This may result in rarer alleles being recorded and this could influence measures such as allelic richness. Sampling four populations at a smaller distance will also increase the chance of sampling the same clone and this needed to be taken into consideration when the data were analysed. These issues occur in any study where the same numbers of samples are taken from populations of different sizes but this is very rarely mentioned in the literature. In this study, it was thought preferable to standardise sample sizes to make analysis easier and to improve comparison between populations. By using a systematic sampling strategy and recording the location of plants, the pattern and intensity of sampling is made explicit. For each plant sampled, a young leaf was removed, cut into 1 cm^2 pieces and placed immediately into a bag containing silica gel to dry. Samples were stored in dry silica gel until further analysis could take place.

2.8.2 DNA extraction

Initially, two methods of DNA extraction were tested using plants from four different populations to determine the best technique for *C. dissectum*. The first was a phenol-chloroform extraction based on Doyle and Doyle (1987): this produced very good quality DNA with 100% amplification using one microsatellite primer (Caca1, n = 24); the method was very economical, but only 48 samples could be extracted over two days. The second method used a DNEasy 96 Plant Kit (Qiagen, Sussex, UK), this allowed extraction of 192 samples per day but was more expensive. Out of the 96 samples extracted using this kit, only 30% amplified successfully, making this method less feasible.

DNA extraction for the 35 leaves from each population therefore used the modified Doyle and Doyle (1987) protocol. 500 µL of hot CTAB (cetyl trimethyl ammonium bromide) extraction buffer containing 2X CTAB buffer and 1% β-mercaptoethanol was added to 2 cm^2 of dried leaf tissue in a sterilised eppendorf tube. (2X CTAB buffer was made using 100 mL Tris-HCl (pH 8), 40 mL 0.5 M EDTA (pH 8), 81.8 g NaCl, 20 g CTAB made up to 1 L with ultra-high purity water). A grinding bead was added to each sample and 24 samples assembled into a TissueLyser mill (Qiagen, Sussex, UK). After grinding for 10 minutes, the samples were vortexed and incubated for 45 minutes in a water-bath at 65 °C. 650 µL of phenol-chloroform were then added to each tube and the samples rocked for 10 minutes to extract proteins. After centrifuging for five minutes at 15000 rpm and 18 °C the upper aqueous phase was pipetted into fresh sterile tubes. 500 µL chloroform-isoamyl alcohol was added to each tube, which, after rocking for 10 minutes to extract more proteins, was centrifuged for five minutes at 15000 rpm and 18 °C. The upper aqueous phase was again removed into fresh sterile tubes, 2/3-volume isopropanol added and the tubes placed in a -20 °C freezer to allow the DNA to precipitate. After 30 minutes, the tubes were centrifuged for five minutes at 15000 rpm and 4 °C; all traces of the supernatant were removed and the resulting pellet of impure DNA allowed to air-dry.

The pellet was re-suspended in 300 µL 1X TE buffer and incubated in a water-bath at 37 °C until the pellet dissolved. 1X TE buffer was made using 10 mL 1 M Tris HCl (pH 8) and 2 mL 0.5 M EDTA (pH 8), made up to 1 L with ultra-high purity water. The DNA was cleaned by adding 20 µL 7.5 M ammonium acetate, followed by 600 µL of ice-cold 100% ethanol. After precipitating in a -20 °C freezer for at least 20 minutes, the tubes were centrifuged for 10 minutes at 15000 rpm and 4 °C. The supernatant was removed and the DNA washed for a final time by adding 400 µL of ice-cold 70% ethanol and centrifuging as before for two minutes. The supernatant was removed; the pellet air-dried and then re-suspended in 50 to 100 µL 1X TE buffer depending on the size of the pellet. Finally, the

DNA samples were treated with 1 μL RNAase (RNase ONE Ribonuclease, Promega). The purity and concentration of the DNA samples was determined using a spectrophotometer and each solution diluted to 0.25 μg μL^{-1} (see section 2.8.1).

2.8.3 Primer screening and PCR

Microsatellite analysis is PCR-based and requires primers that are complementary to the two flanking regions of the microsatellite loci (Beebee & Rowe 2004). The nine primer pairs developed by Jump *et al* (2002) for *C. arvense* were tested for use in *C. dissectum.* For each primer pair, six *C. dissectum* individuals from different populations and a water control were amplified and the products visualised on 1% agarose gel using electrophoresis.

One primer (Caca17) showed very inconsistent amplification, so was discarded. The remaining primers showed some inconsistency in amplification, so the annealing temperature, concentration of magnesium chloride and the PCR additives BSA (bovine serum albumin), DMSO (dimethylsulfoxide) and Tween-20 (a non-ionic detergent) were varied empirically in order to determine the optimal PCR conditions for each primer pair for *C. dissectum.* **Table 2.4** shows the primers used and the optimal cycling conditions determined. The final PCR reaction mixture was: 1 μL template DNA (0.250 μg μL^{-1}) in a total volume of 10 μL containing 5 μL 2X Thermo-Start PCR Master Mix with 1.5/2.0 mM magnesium chloride (ABgene), 0.05 μL DMSO, 1 μL each of the forward and reverse primer (5 μM) and 1.95 μL of ultra high purity water. PCR was performed in a PTC-100 thermocycler (MJ Research).

Table 2.4 Primer sequence and cycling conditions for microsatellite primers (developed for *Cirsium acaule* by Jump *et al.* (2002) and optimised for use in *C. dissectum*. (F: = forward, R: = reverse).

Locus	Primer sequence and modification at 5' primer end (5'-3') for *C. dissectum*. Florescent labels: L = black, F = blue, E = green.	Cycling conditions for *C. acaule* (Jump 2002)		Cycling conditions for *C. dissectum*	
		Program	$MgCl_2$ (mM)	Program	$MgCl_2$ (mM)
Caca1	F: GTTTCTT- TTT GAA GTG GAT CTT CGC ACG R: F- CAT GGG AGA CGA ACT AAC AGA TGC	64	2.5	64	2.0
Caca4	F: GTTTCTT- ATC ACC GCT TCC ACC GTC TC R: E- GCT TAT TAG AAC CGC CAT TGA AAG C	65/55TD	2.5	63	2.0
Caca5	F: E- ACC CAA CCC TCG ATC TGA A R: GTTTCTT- GAG GAT ACC GGC GAT TGT TA	62/52TD	1.5	60	1.5
Caca7	F: F- CCC AAA CTC CCA CCT TCA TTT G R: GTTTCTT- GTC GGA GAT GCT CCG GTG AC	64	2.5	64	2.0
Caca10	F: GTTTCTT- GAA TTC GCG ACA ACA CAC GC R: L- GGT AAG GAA TGA ATG ATT GGG CTC	65/55TD	1.5	63	1.5
Caca16	F: L- TCG TGC TCT TCG ATT GAT TG R: GTTTCTT- CAG AAA ACC GCT CCA TTG C	60	2.5	60	2.0
Caca22	F: F- GGC TCT GCC TCA CCC ATC TC R: AGG TGT TCA GCA CGG TTC GG	65/55TD	1.5	65	1.5
Caca24	F: F- TGG ATA ACG CGC TAG ATC AC R: GTTTCTT- AAG AAC TCA ATT AGT AGG AAG TGG	62/52TD	2.5	55	2.0
PCR programs (ex-Jump 2002) 55: (95°C 3min): 35 cycles of (94°C 30s, 55°C 30s, 72°C 30s): (72°C 10m) 60: (95°C 3min): 35 cycles of (94°C 30s, 60°C 30s, 72°C 30s): (72°C 10m) 64: (95°C 3min): 35 cycles of (94°C 30s, 64°C 30s, 72°C 30s): (72°C 10m) 62/52TD: (95°C 3min): 5 cycles of (94°C 30s, T°C 30s, 72°C 30s, where T drops from 62 to 54 in 2°C steps): 15 cycles of (94°C 30s, 52°C 30s, 72°C 30s): (72°C 10m) 65/55TD: (95°C 3min): 5 cycles of (94°C 30s, T°C 30s, 72°C 30s, where T drops from 65 to 57 in 2°C steps): 15 cycles of (94°C 30s, 55°C 30s, 72°C 30s): (72°C 10m)					
PCR programs for *C. dissectum* (optimised by author) 55: (95°C 15min): 35 cycles of (94°C 30s, 55°C 30s, 72°C 30s): (72°C 10m) 60: (95°C 15min): 35 cycles of (94°C 30s, 60°C 30s, 72°C 30s): (72°C 10m) 63: (95°C 15min): 35 cycles of (94°C 30s, 63°C 30s, 72°C 30s): (72°C 10m) 64: (95°C 15min): 35 cycles of (94°C 30s, 64°C 30s, 72°C 30s): (72°C 10m) 65: (95°C 15min): 35 cycles of (94°C 30s, 65°C 30s, 72°C 30s): (72°C 10m)					

Once primers were amplifying successfully, each locus was tested to see whether it was polymorphic in *C. dissectum.* In order to do this, PCR products were visualised using a CEQ 8000 Genetic Analysis System (Beckman Coulter). This system required one of each primer pair to be fluorescently labelled in order to visualise the products. To decide which primer in each pair to label, reaction sets were set up using only the forward primer, or only the reverse primer and one using both forward and reverse. Products were visualised on 1% agarose gels. Of the forward + forward and reverse + reverse reactions, those giving no product or the least defined product were chosen to be fluorescently labelled, as these will yield a clearer product with alleles that are easier to score. In addition there are three colours that can be used to label each primer allowing multiplexing of the PCR products from different primers. Labels were chosen so that products from different primers that

were of similar size were labelled with different colours. In this way it was possible to set up two multiplexes, one consisting of Caca 1, 5, 7 and 10 and the other of Caca 4, 16, 22 and 24. The sequence: 5'GTTTCTT3' was added to the 5' end of the unlabelled primer from each pair to reduce genotyping error due to variable adenylation during PCR (Jump 2002). The only exception to this was Caca22, where the addition of the 5'GTTTCTT3' sequence was found to reduce amplification. All of the primers proved to be polymorphic in *C. dissectum.*

2.8.4 Identifying alleles

The fragment analysis module, within the CEQ 8000 Genetic Analysis System software, sized the PCR products and provided a facility for automatically labelling the different sized peaks as different alleles. This required a process of 'allele binning' to be performed using a number of individuals to help determine what were alleles and what were PCR artefacts such as stutter peaks. In order to do this and also to check the consistency of "allele calling", 50 individuals were amplified twice with all eight primers. Automatic allele binning was effective although every individual needed to be checked manually as failed amplifications sometimes led to spurious peaks being labelled as alleles. With automatic binning and manual checks, consistency between the alleles was judged to be very good (**Table 2.5**).

Out of the 770 leaves collected, 16 would not amplify for any of the loci. The DNA pellets of these individuals were often tinged with red rather than white and it is likely that contamination with secondary compounds prevented successful PCR. Of the remaining 754 individuals, some did not amplify with particular primers; failed amplifications were carried out a further two times before discounting that individual for that primer (**Table 2.5**).

Table 2.5 Correspondence between allele calling when 50 individuals were amplified twice and the total number of plants successfully genotyped using 8 microsatellite loci.

Locus	Correspondence between 2 replicates % (n = 50)	Percentage of individuals successfully genotyped (n = 754)
Caca 1	100	95
Caca 4	100	96
Caca 5	99	98
Caca 7	100	98
Caca 10	100	91
Caca 16	99	97
Caca 22	98	94
Caca 24	100	97

2.8.5 Null alleles and linkage disequilibrium

Microsatellites can suffer from null alleles; this is where a deletion or point mutation in the primer binding site of a specific allele interferes with priming resulting in that allele not being amplified (Schlötterer 1998). The presence of null alleles is suspected if there is a surplus of homozygotes; however such deviations from Hardy-Weinberg equilibrium may also be due to other factors, such as inbreeding. If this is the case, however, then all of the loci should be similarly affected. An excess of homozygotes for a particular locus may therefore indicate the presence of null alleles (Schlötterer 1998). GENETIX v.4.02 (Belkhir *et al.* 2001) was used to determine the observed and expected heterozygosity for each locus and population. For all of the loci, across all populations, there tended to be an excess of homozygotes resulting in lower observed heterozygosities (see Chapter 4). For Caca10, however, there was a particularly large excess of homozygotes for populations ILG, IBL, IGC, ILT and IDL, so this locus was removed from all further analyses.

Another potential problem with microsatellite loci is linkage disequilibrium caused by two loci being present on the same chromosome; this leads to alleles from different loci being associated with each other more commonly than expected by chance (Lowe *et al.* 2004). FSTAT v2.9.3.2 2002 (Goudet 2001) was used to investigate linkage disequilibrium. At the 0.1% nominal level with 210,000 permutations, there was no

significant linkage disequilibrium between any combinations of primers, so this was discounted as a potential complicating factor.

Chapter 3

3 Site characteristics, plant communities and reproductive biology of *Cirsium dissectum.*

3.1 Introduction

The previous research described in Chapter 1 highlighted the response of *C. dissectum* to the effects of nutrients and increases in site productivity. As fertilisers are added, or site productivity increases through succession, *C. dissectum* is unable to compete with species that are able to build up biomass more quickly e.g. *Molinia caerulea. C. dissectum* responds by showing a greater turnover of rosettes and a greater proportion of flowering rosettes (Jongejans *et al.* 2006a; Jongejans *et al.* submitted). Information on nutrient levels and indicators of site productivity are therefore important habitat variables to record and may relate to plant fitness and genetic diversity.

The review of *C. dissectum* in Chapter 1 also indicated two areas that could warrant further research, in order to build a complete picture of the ecology of this species. Firstly,

C. dissectum has been described from a range of wet grasslands, heaths and fens throughout the British Isles (Braun-Blanquet & Tüxen 1952; Ivimey-Cook & Proctor 1966; Wheeler 1980; White & Doyle 1982; Rodwell 1991; 1995; Blackstock *et al.* 1998; Yeo *et al.* 1998). It can also occur in dune slacks but the plant species with which it is associated in this habitat have not been reported.

Secondly, further research is required into the reproductive biology of *C. dissectum* in the British Isles. Ross (1999) investigated germination conditions and found that germination was greatest for stratified seeds at 31 °C but that germination could occur in a variety of conditions. Seedling establishment however was low when seeds were added to field sites (Isselstein *et al.* 2002; Jongejans *et al.* 2006b; Smulders *et al.* 2000; Soons *et al.* 2005) and seedlings were very rarely observed in field conditions (Kay & John 1994; Jongejans *et al.* 2006b). Population dynamics suggests that clonal propagation is the dominant form of reproduction, as sexual reproduction is limited by low seedling recruitment rates in established vegetation (Jongejans 2006b; Jongejans *et al.* submitted). Seed dispersal has been measured directly by Kay & John (1994) in a Welsh rhos pasture and indirectly using mechanistic models (Soons & Heil 2002 and Soons 2006). This shows that most seeds disperse close to the parent plant with dispersal distances over 20 m being rare but possible under stormy weather conditions. There is no basic description, however, of the morphology of the reproductive organs in *C. dissectum* and seed production, germination and survival have not been measured over a range of sites in the British Isles. In addition, *C.* x *forsteri* is described as the commonest hybrid thistle (Stace 1997; Preston *et al.* 2002) but hybridization levels have not been recorded. Hybridization can have negative effects on rare plants leading to genetic assimilation, outbreeding depression or competition between hybrids and parents (Levin *et al.* 1996); alternatively, it can have a positive effect, introducing novel alleles (Rieseberg 1991).

Population dynamics (Jongejans *et al.* submitted) emphasised the importance of clonal reproduction in *C. dissectum* but little is known about the number, distribution or age of clones. It is not possible to identify individual clones in the field, so the number of genets within a population cannot be determined. Rosettes often grow in definite patches that may represent single clones, but equally, the relatively long rhizome length of *C. dissectum* means that intermingling between genets is possible. Asexual reproduction has consequences for sexual reproduction through altering levels of geitonogamy (pollination between flowers of the same plant) (Charpentier 2002). If a clone is composed of numerous ramets, this is likely to increase geitonogamous mating between different capitula belonging to the same clone. In self-incompatible species this may lead to a reduction in seed set and pollen wastage and in self-compatible species can increase levels of inbreeding. The pattern of clonal growth will also influence geitonogamy levels, as clones with intermingling ramets are more likely to outcross (Charpentier 2002).

Molecular markers can be used to identify individual clones but caution is needed, as the resolution of the marker will affect the number of different genotypes distinguished (McLellan *et al.* 1997). This has been illustrated in the previous genetic studies conducted on *C. dissectum*; when allozymes were used many populations shared the same multilocus genotype (Kay & John 1994), whilst when AFLP was used, no identical multilocus genotypes were found (M. Smulders pers. comm.). Parks & Werth (1993) developed a measure that can be used to determine the power of molecular markers for clone identification. This measure (P_{gen}) gives the probability that two genets would have the same genotype by chance, and so provides an estimate of the likelihood that the genotype could have arisen twice through sexual recombination (Parks & Werth 1993; Widén *et al.* 1994). The probability determined, however, assumes no somatic mutation, random mating and no linkage among loci; the first two of these are unlikely to be met in natural populations. Even with these limitations, P_{gen} is frequently used (Reusch *et al.* 2000;

Stehlik & Holderegger 2000; Suvanto & Latva-Karjanmaa 2005; Reusch 2006), whilst other studies simply assume that all samples with the same multilocus genotype belong to the same clone (Kudoh *et al.* 1999; Brzosko *et al.* 2002). Determination of clonal structure also varies depending on the spatial scale examined, with smaller scales revealing the presence of more clones (McLellan *et al.* 1997). Despite this observation, many studies do not state the sampling strategy used (Widén *et al.* 1994).

This chapter examines various aspects of the ecology of *C. dissectum.* Specifically it describes the characteristics of sites throughout the British Isles including soil nutrients, bare soil, vegetation height and management. It then describes the range of plant communities within which *C. dissectum* is found in Britain, including wet grasslands, heaths, fens and dune slacks. The reproductive biology of *C. dissectum* is examined by describing plant phenology, morphology of sexual and vegetative reproductive organs, seed production, germination, dispersal, animal feeders and pollinators. The number of flowering *C.* x *forsteri* in the populations surveyed is recorded and *C.* x *fosteri, C. palustre* and *C. dissectum* genotyped to investigate whether microsatellite markers can be used to identify hybrid plants. Finally, the number, distribution and age of clones in different populations are described.

3.2 Methods and data analysis

General methods are described in Chapter 2 and a summary provided here.

3.2.1 Site characteristics

For each of the 22 sites, five topsoil samples were taken with an auger (depth 14 cm, diameter 3 cm). The top and bottom halves of the soil core were separated and the top and

bottom samples pooled for the site. Samples were air-dried and pH, organic matter, total Kjeldahl nitrogen, phosphorus, calcium and potassium determined. The mean of the top and bottom soil fractions was used for each soil measure if their bivariate correlation was greater than 0.7 (Tabachnick & Fidell 2007); as a result, means were used for all soil measures except phosphorus. The mean vegetation height and percentage cover of bare soil was determined in each of 30 1 x 1 m quadrats. Soil samples were taken and vegetation height and bare soil recorded for all of the 22 sites over 19 days in July 2004. Where possible, the management of the site was also recorded after speaking to land owners and managers.

3.2.2 Plant communities

Eleven sites in England and Wales were surveyed using 10 2 x 2 m quadrats and MAVIS Plot Analyser v.1 (Smart 2000) and Rodwell (1991; 1995; 2000) used to assign the sites to National Vegetation Classification (NVC) communities. Detrended correspondance analysis (DCA) was performed to examine the relationships between quadrats and sites using the program DECORANA within the Community Analysis Package 2.15 (Pisces Conservation 2003).

3.2.3 Reproductive biology

Five *C. dissectum* sites were visited throughout the seasons, from 2002 to 2006 and botanical observations recorded including plant morphology, phenology and animal visitors. Capitula were dissected from a number of sites to observe floral biology and seeds were grown so that seedling morphology could be recorded. Light microscopy was used to observe pollen grains. Seed dispersal was measured on 110 seeds with pappus attached over two days at Braunton Burrows (EBB). On both days wind speed was approximately 7 to 8 m s^{-1} at 10 m height, with occasional stronger gusts. Capitula containing ripe seeds

ready for dispersal were located, and seeds with pappus attached gently dislodged and tracked.

For each of the 22 *C. dissectum* sites, population area, density and the number of flowering and vegetative rosettes were recorded and 30 seed heads collected to determine seed production, germination and survival. The number of predated seed heads and the proportion of seeds affected were counted. Each seed head was stored individually in a paper bag and many of the invertebrates living within the seed heads were preserved. In particular, Tephritid flies that were pupating within the seed heads continued to develop and emerged as adults; all species found within the seed heads were identified by C. Woolley (University of Plymouth).

Each site was surveyed for the presence of flowering *C.* x *forsteri.* Leaf samples of two *C.* x *forsteri* plants were collected from Kenfig (WKF), along with the closest *C. dissectum* and *C. palustre* plants; these were genotyped using seven microsatellite loci.

To determine the pattern of clonal growth within *C. dissectum,* 35 leaves were sampled from each population and genotyped using seven microsatellite loci. A systematic sampling strategy was used: for 18 populations plants were sampled every 10 m along a transect with subsequent transects 10 m apart, in three populations plants were sampled every 5 m along a transect with a 10 m distance between transects and the smallest population, Wicken Fen (EWF), was sampled on a 2.5 x 2.5 m grid. In EWF, the size and position of every patch of *C. dissectum* rosettes was also recorded. Samples sharing identical multilocus genotypes (IMLGs) were identified using a computer program written by C. Ford (Spirent Communications plc, source code available on request) and P_{gen} calculated for each IMLG to determine the probability that the same genotype could have arisen through sexual recombination (Parks & Werth 1993, modified by Widén *et al.* 1994).

$$P_{gen} = \left(\prod_{i=1}^{N} p_i q_i \right) 2^h$$

Equation 3.1 where p_i and q_i is the frequency of the two alleles at the ith locus of the N loci in the population and h is the number of heterozygous loci represented in the genotype.

The proportion of distinguishable genotypes (number of genotypes / number of samples) was calculated to provide a measure of genotypic diversity (McLellan *et al.*1997) and the maximum distance between IMLGs determined as a measure of maximum clone size.

3.3 Results

3.3.1 Site characteristics

Appendix A contains descriptions for all of the sites and Appendix B the values for soil nutrients, vegetation height, bare soil and management. Values typical of other semi-natural grasslands and an improved pasture are provided for comparison. The most common form of management was summer grazing using cattle and this was observed in eight of the sites including all of the British rhos pastures. Four sites were also grazed but for greater duration, with stock kept on the site for more than half of the year. This included the New Forest heath sites that were grazed with ponies and cattle and some of the Irish pastures. Lough Talt (ILT), the two dune slacks Kenfig (WKF) and Braunton Burrows (EBB) and Wicken Fen (EWF) were all cut, although the frequency was variable between sites. For two of the sites, there was no evidence of management and the position of the sites would make grazing difficult, leading to the conclusion that no active management was taking place. Finally, management was uncertain for four of the sites. This included the two sites on the Burren, Lough Bunny (ILB) and Lough Gealain (ILG).

The traditional management in this area would be to use these grasslands as winterage (Dunford 2002) but it was not possible to confirm this. Management was therefore divided into five categories: none, summer grazing (defined as grazing for less than six months of the year), continuous grazing (grazing for six months of the year or more), cut and unknown. These categories were used in Chapter 4 to investigate the effect of site management on the habitat variables measured.

Phosphorus levels were low throughout all of the sites, whilst there was more variation in levels of potassium, calcium and organic matter, pH varied from acidic (minimum 4.5) to slightly acidic (maximum 6.1) (**Table 3.1**). Sites showed variation in vegetation height from 107 to 833 mm, whilst the amount of bare soil varied from 0 to 20%.

Table 3.1 Summary of soil nutrient levels for 22 *Cirsium dissectum* sites throughout the British Isles, sampled in July 2004. (Mean with standard deviation in parentheses is given along with minimum and maximum values with site code. The values for each site are presented in Appendix B).

Nutrient	Mean (SD) N = 22	Minimum Site	Maximum Site
Total (Kjeldahl) N %	0.7 (0.6)	0.1 WKF	2.4 EWF
P mg kg^{-1} 0-7 cm	3.7 (4.1)	0 EAC, EKS, IDL, IMA, WKF	12.4 WDB
P mg kg^{-1} 7-14 cm	1.4 (1.1)	0.5 IDL	5.1 IAR
K mg kg^{-1}	119 (107)	18 ILT	529 IGC
OM %	31 (26)	6 WKF	87 IAR
Ca mg kg^{-1}	3185 (3583)	248 EMM	12112 IBL
pH	5.2 (0.6)	4.5 ERW	6.1 ILB
Bare soil %	7 (7)	0 EMM, EWF, IAR, IME	20 EMO, ERW, IDL
Vegetation height mm	364 (179)	107 ILL	833 EWF

3.3.2 Plant communities

Appendix C lists the plant species and their abundance within each of the 11 sites surveyed, detrended correspondence analysis was used to produce an ordination plot of these data (**Figure 3.1**). Three broad groups emerged when all 11 sites were included in the analysis (**Figure 3.1**a):

a) The sand dune slacks at Braunton Burrows (EBB) and Kenfig (WKF). These were classified as SD14b and SD14d (*Salix repens - Campylium stellatum* dune-slack, *Rubus caesius – Galium palustre* and *Festuca rubra* subcommunties).

b) The fen vegetation at Wicken Fen (EWF) that was classified as S24c (*Phragmites australis – Peucedanum palustre* tall-herb fen, *Symphytum officinale* subcommunity).

c) The remainder of sites that consisted of the heaths, Aylesbeare Common (EAC), Marlpitt Oak (EMO) and Rans Wood (ERW) that were all classified as M16b (*Erica tetralix – Sphagnum compactum* wet heath, *Succisa pratensis – Carex panicea* subcommunity) and the rhos pastures including Knowstone Moor (EKS), Meshaw Moor (EMS), Mambury Moor (EMM), Baddesley Common (EBC) and Drostre Bank (WDB). These were all classified as M24c (*Molinia caerulea – Cirsium dissectum* fen meadow, *Juncus acutiflorus – Erica tetralix* subcommunity) except WDB that was closest to M24.

As the dune slacks and fen were very different from the rhos pastures and heaths these first two groups were removed to see if this revealed more differentiation in the remaining sites; the M16b, M24 and M24c sites showed some differentiation but still overlapped within the ordination plot (**Figure 3.1**b).

The characteristic species found with *C. dissectum* within the rhos pastures (M24, M24c) and heaths (M16b) were *Molinia caerulea, Erica tetralix, Juncus acutiflorus, Carex panicea, Succisa pratensis, Lotus pedunculatus* and *Potentilla erecta.* These were replaced in the dune slacks (SD14b, SD14d) by *Agrostis stolonifera, Carex nigra, Equisetum palustre, Mentha aquatica, Hydrocotyle vulgaris, Lotus corniculatus, Epipactis palustris, Ranunculus acris* and *R. flammula.* The tall herb fen (S24c) was dominated by *Phragmites australis, Juncus subnodulosus, Filipendula ulmaria, Thalictrum flavum, Symphytum*

officinale, Iris pseudoacorus and *Angelica sylvestris* with *Molinia caerulea* and *Carex panicea* also frequent within this community (see Appendix C).

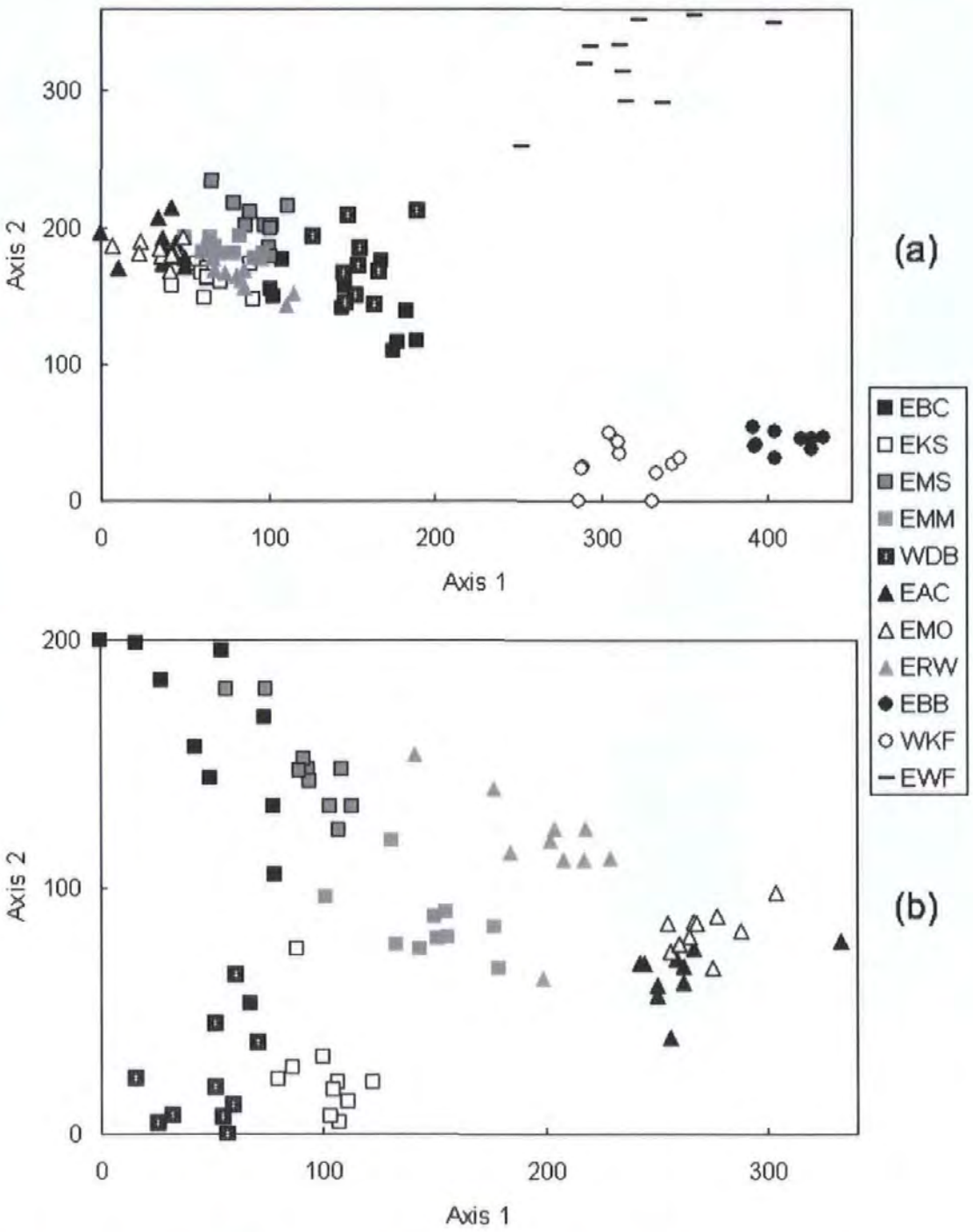


Figure 3.1 Detrended correspondence analysis of sites where *Cirsium dissectum* is present. (Each data-point represents a single quadrat. The shape of each data-point represents the National Vegetation Classification community that the site belongs to: Squares M24c; squares containing a star M24; triangles M16b; closed circles SD14b; open circles SD14d; dashes S24c. (a) Analysis of all 11 sites surveyed. (b) The same analysis with the SD14b, SD14d and S24c communities removed).

3.3.3 Reproductive biology

3.3.3.1 *Phenology*

In all of the sites observed in winter and in common garden conditions, the large elliptical-lanceolate leaves of *C. dissectum* died back in autumn and were often replaced with much smaller, lanceolate leaves. These were hairless and fleshy and persisted throughout the winter, often being partially or completely submerged in standing water (**Plate 3.1**). The larger, hairy leaves, began to appear again by March, with full-sized rosettes present by May. Across all 22 populations, flowering started as early as the end of May, with the greatest number of capitula seen in June and the beginning of July, with flowering continuing throughout July. Ripe seed heads were seen throughout July and August.

Plate 3.1 Overwintering leaves of *Cirsium dissectum* at Braunton Burrows (EBB).

3.3.3.2 *Morphology and sexual reproduction*

C. dissectum has a hemi-crytophyte basal rosette growth form. It typically produced new rosettes at the end of long rhizomes but new rosettes were also seen at the base of existing ones. Rhizomes were a pale-straw colour and smooth, with small brown scales at the nodes. Rhizome lengths were variable, from close to the parent plant to up to c. 40 cm; after two years, plants had produced a large caudex, approximately 10 x 2 cm.

Appendix D lists the reproductive features of plants from all 22 populations and these are summarised in **Table 3.2**. There was considerable variation in the proportion of

rosettes that flowered at different sites; some populations were almost entirely vegetative, whilst during July in Kenfig (WKF), 23% of all rosettes flowered.

Dissection of a number of florets showed that the corolla consisted of a tube c. 9 mm long and a limb of c. 11 mm, which divided into five irregular lobes of c. 5 mm. The corolla tube was white and the limb a deep magenta-purple. Five epipetalous stamen were attached at the junction of the tube and limb of the corolla; the filaments were c. 5 mm long and the anthers were c. 6 mm long, connate and creamy-white in colour. The anther tube enclosed the central part of the style and ended in five teeth. The style was magenta-purple and c. 25 mm long (**Figure 3.2**). Florets had a sweet perfume and copious nectar. The pollen grain was circular to three angled in polar view with a diameter of c. 50 μm; in meridian view, it was echinate and circular to slightly elliptical (**Figure 3.3**).

For all 22 populations, the mean number of hard seeds within each seed head was 34, this was highly variable however, the minimum was seven seeds per seed head (Kenfig (WKF)) and the maximum 83 seeds (Aylesbeare Common (EAC)) (**Table 3.2**). Germination under standard conditions (see section 2.5) was low, with a mean of 9%; seedling survival was higher with 27% of seedlings surviving. Cotyledons were obovate, with the tip rounded 12 to 35 x 5 to 8 mm. The first true leaves were elliptical-lanceolate to spathulate, having some hairs above and soft prickles, cottony below (**Figure 3.2**).

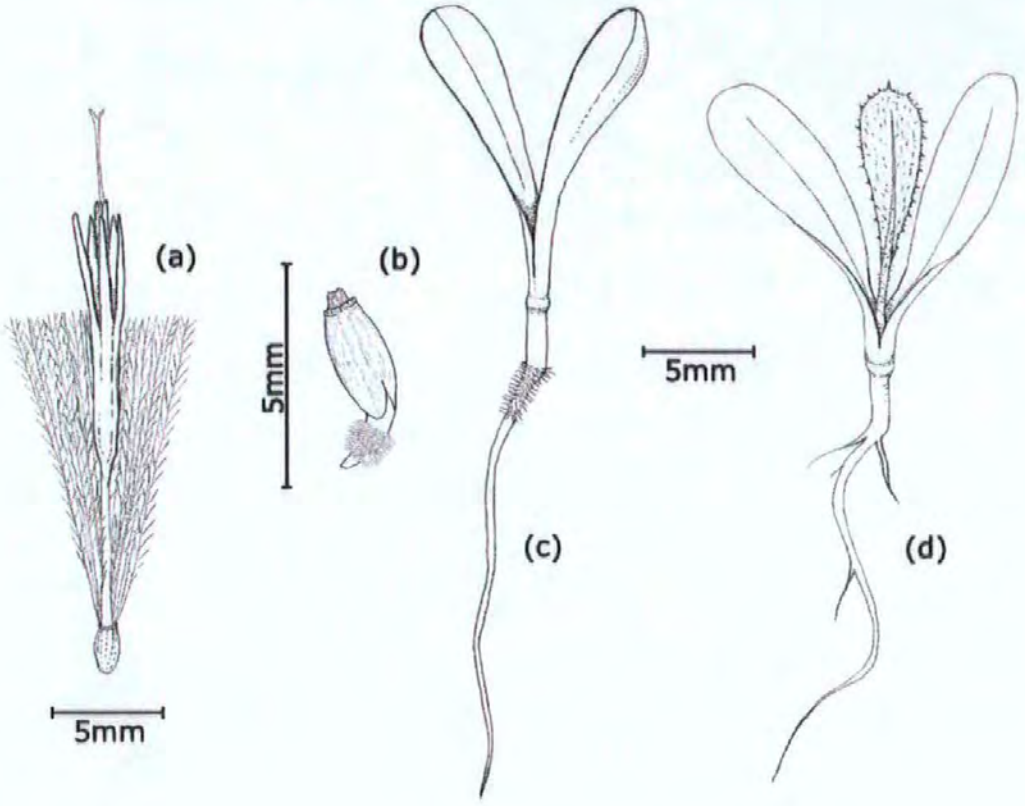


Figure 3.2 Floret and seedling development in *Cirsium dissectum*: (a) single floret with part of the pappus removed, (b-d) developing seedling, (illustrations by author).

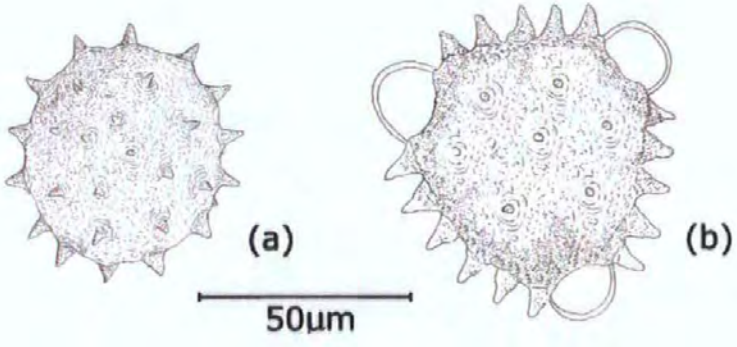


Figure 3.3 Pollen grain in (a) meridian and (b) polar view, (illustrations by author).

Table 3.2 Summary of reproductive characters for 22 populations of *Cirsium dissectum* sampled in July 2004. (Mean with standard deviation in parentheses is given along with minimum and maximum values with site codes. The values for all 22 sites are presented in Appendix D. The density measure provided only used quadrats that contained *C. dissectum*).

	Mean (SD) N = 22	**Minimum Site**	**Maximum Site**
Population area m^2	20365 (26107)	981 EWF	98935 EMO
Density of rosettes m^2	11 (5.54)	4 IAR, WCG	24 EBB
Total number of rosettes	178231 (305013)	3138 EWF	1184757 IGC
Number of flowering rosettes	1308 (1816)	19 IMA	5500 IGC
Proportion of flowering rosettes %	4.7 (5.3)	0 EMO, ILL	23.0 WKF
Number of hard seeds per capitulum	33.6 (19.4)	6.50 WKF	82.7 EAC
Seed mass mg	2.62 (0.57)	1.27 ERW	3.63 IAR
Germination %	8.6 (8.8)	0 ERW, ILL,	29.3 IAR
Seedling survival %	27.1 (22.6)	0 EBB, EMO, IMA, WKF	69.2 IME
Number of seed heads predated %	9 (14)	0 EAC, EMO, ERW, IBL, IGC, ILG, ILL, IMA	63 ILB

3.3.3.3 *Seed dispersal*

Seeds are attached to a pappus for dispersal but field observations showed that the pappus often became detached before the seeds left the capitulum. In wet conditions the pappi stuck together preventing dispersal. As the rosette began to die, the flowering stem tended to wither and eventually break just below the capitulum. This may be an additional dispersal mechanism for the seeds still trapped inside (**Plate 3.2**). 55% of seeds landed within 1 m of the parent plant and only one seed (0.9%) travelled over 20 m (**Figure 3.4**).

Plate 3.2 Left: seed dispersal in *Cirsium dissectum*. Right: Withering of the stem below the seed head.

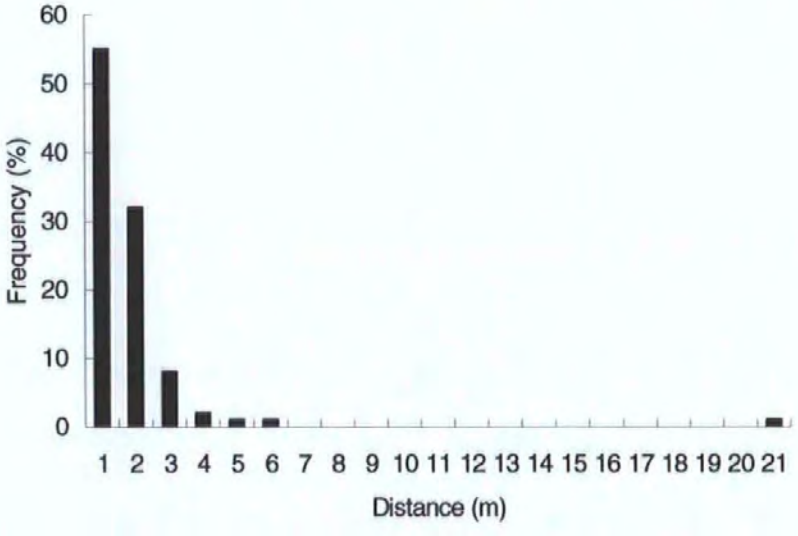


Figure 3.4 Dispersal distances (m) for 110 seeds from 11 capitula of *Cirsium dissectum*. (Ripe seeds with pappus attached were dislodged from capitula and tracked. If, after landing, the seed did not move for 2 minutes, the distance it had travelled was measured).

3.3.3.4 *Seed predation and insect visitors*

Fourteen out of the 22 populations showed some signs of predation when the seed heads were examined (Appendix D). The effects varied from small holes created in some of the seeds, whilst the rest of the seeds in the capitulum were unaffected, to the complete destruction of all the developing seeds. The seed heads of 13 out of 22 populations contained adult Tephritid flies and it is therefore likely that, as larvae, they caused most of the predation observed (**Table 3.3**). The small holes seen in the seeds were consistent with predation by these flies (Redfern 1968). The highest levels of predation were recorded at Lough Bunny (ILB) with 63% of capitula showing some sign of predation. *Chaetostomella cylindrica* adults were found in 48% of the bags in which seed heads had been collected from Lough Bunny, suggesting that this was the cause of most of the predation. *C. cylindrica* was the most widespread species, occurring in seed heads throughout the British Isles, *Terellia ruficauda* and *Terellia serratulae* were found only in seed heads from England and Wales, whilst *Tephritis conura* was found only in seed heads from Ireland.

A range of insects were recorded visiting *C. dissectum* flowers and it is likely that the Hymenoptera, Lepidoptera and *Volucella pellucens* were all potential pollinators.

Table 3.3 Invertebrate species recorded from 22 *Cirsium dissectum* sites within the British Isles.

Diptera: Tephritidae		**Population codes and the % of seed heads in which adult flies were found**									
	*Chaetostomella cylindrica*	EKS 3	EMM 20	EMS 23	IAR 7	IDL 3	ILB 48	ILT 7	WDB 3	WKF 7	WMM 13
	*Tephritis conura*	IAR 3	IDL 3	ILT 27	IME 7						
	*Terellia ruficauda*	EBB 5	EMM 3	EMS 3	EWF 4	WDB 7	WKF 3				
	*Terellia serratulae*	EWF 9	WKF 7								
Other invertebrates found within seed heads		Hymenoptera: chalicid & braconid wasps Lepidoptera: moth larvae, pupae & adults Coleoptera: *Sitona* sp. Acari: orabatid mite Araneae: *Tibellus oblongus*									
Insect species visiting flower heads or observed on *C. dissectum*		Diptera: *Volucella pellucens* Hemiptera: *Philaenus spumarius* Hymenoptera: *Bombus pascuorum* Lepidoptera: *Eurodryas aurinia, Argynnis aglaja, Gonepteryx rhamni, Zygaena trifolii*									

3.3.3.5 *Hybridization in Cirsium dissectum*

Cirsium x *forsteri* (**Plate 3.3**) was recorded in flower at Kenfig (WKF) and Wicken Fen (EWF), plants always being found in close proximity to *C. dissectum* and *C. palustre*. Some areas of WKF had large vegetative patches with very variable leaf morphology (**Plate 3.3**); it seems likely that these were composed of *C. dissectum* and *C.* x *forsteri*.

Two seed heads were collected from *C.* x *forsteri* at EWF. One contained three hard seeds with a mean seed mass of 4.78 mg, one of these seeds germinated but did not survive as a seedling. The other seed head contained 13 hard seeds with a mean seed mass of 3.83 mg, none of these seeds germinated.

Genotyping two *C.* x *forsteri* plants, along with *C. dissectum* and *C. palustre* (from WKF), revealed that *C.* x *forsteri* could be distinguished from *C. dissectum* and *C. palustre*

using microsatellite loci (**Table 3.4**). The different multilocus genotypes found in the two hybrids suggests that these plants were not clones and had arisen from two separate seeds.

Plate 3.3 Hybridization in *Cirsium dissectum*. Top right: *C.* x *forsteri* growing at Kenfig (WKF) (07/06/03). Top left: *C.* x *forsteri* collected from WKF. Bottom right: the range of leaf morphology in vegetative rosettes of *C. dissectum* and *C.* x *forsteri* at WKF (08/06/02).

Table 3.4 Genotypes of *Cirsium dissectum*, *C. palustre* and two *C.* x *forsteri* plants using seven microsatellite loci. (Alleles in black are present in *C. dissectum*, alleles in blue are not found in any *C. dissectum* plants from any population sampled).

Locus	Alleles present			
	C. dissectum	***C. palustre***	***C.* x *forsteri* 1**	***C.* x *forsteri* 2**
Caca1	235:237	239:239	237:237	No amplification
Caca4	105:105	***122:122***	***122:122***	***122:122***
Caca5	169:171	***165:165***	***165***:169	***165***:175
Caca7	154:154	138:***140***	148:148	148:148
Caca16	129:129	***125:125***	***125***:129	***125***:129
Caca22	205:205	201:201	203:203	201:201
Caca24	230:236	***222:222***	***222***:230	***222***:236

3.3.3.6 *Asexual reproduction in Cirsium dissectum*

Clone maps for all of the populations where identical multilocus genotypes (IMLGs) were found are presented in Appendix E. All P_{gen} values were below 0.05, so individuals with the same multilocus genotype were considered to be members of the same clone (**Table 3.5**). As expected, the proportion of distinguishable genotypes (G/N) was lower in the populations that were sampled at a smaller spatial scale. When the proportion of distinguishable genotypes for these populations was adjusted to the 10 x 10 m spatial scale, G/N increased. For most populations, the proportion of distinguishable genotypes and therefore genotypic diversity was high. Braunton Burrows (EBB) was an exception, with a G/N of 49% at 5 x 10 m and 65% at 10 x 10 m. In Wicken Fen (EWF) (**Figure 3.5**), nearly all of the patches of *C. dissectum* contained many different clones and EBB (Appendix E) showed that different clones intermingled within the population.

Table 3.5 Number of ramets and genets found within *Cirsium dissectum* populations and the proportion of distinguishable genotypes (G/N) (this is a measure of genotypic diversity). (G/N values in parentheses for Braunton Burrows (EBB), Wicken Fen (EWF), Meencargagh (IME) and Welsh Moor (WWM) were adjusted for a 10 x 10 m spatial scale. Maximum length of identical multilocus genotypes (IMLGs) is given and in parentheses the probability of that genotype arising through sexual recombination (P_{gen}). The range of P_{gen} values for all IMLGs found is also included).

Popn.	Ramets (N)	IMLG (genets) (G)	Sampling strategy	G/N*100	Max. IMLG length (m) with P_{gen} values in parentheses	P_{gen} range for all IMLG
EAC	35	35	10 x 10	100.00		
EBB	35	17	5 x 10	48.57 (64.71)	75 (0.037)	0.0234 - 0.0490
EKS	34	32	10 x 10	94.12	10 (0.0001 - 0.0012)	0.0001 - 0.0013
EMA	35	34	10 x 10	97.14	10 (0.0001)	0.0001
EMO	32	28	10 x 10	87.50	10 (4.21×10^{-6} - 0.0004)	4.21×10^{-6} - 0.0004
EMS	34	34	10 x 10	100.00		
ERW	34	33	10 x 10	97.06	10 (3.02×10^{-6})	3.02×10^{-6}
EWF	35	27	2.5 x 2.5	77.14 (100)	10 (0.0232)	0.0011 - 0.0232
IAR	35	32	10 x 10	91.43	70 (7.75×10^{-5})	7.75×10^{-5} - 0.0201
IBL	35	35	10 x 10	100.00		
IDL	34	33	10 x 10	97.06	10 (1.19×10^{-5})	1.19×10^{-5}
IGC	35	28	10 x 10	80.00	20 (0.0007 - 0.0075)	0.0007 - 0.0054
ILB	33	33	10 x 10	100.00		
ILG	35	33	10 x 10	94.29	70 (0.0085)	0.0029 - 0.0085
ILL	35	35	10 x 10	100.00		
ILT	35	35	10 x 10	100.00		
IMA	34	31	10 x 10	91.18	50 (0.0023)	0.0002 - 0.0023
IME	35	25	5 x 10	71.43 (94.44)	100 (0.0036)	8.40×10^{-5} - 0.0048
WCG	34	34	10 x 10	100.00		
WDB	33	31	10 x 10	93.94	80 (0.0086)	0.0086 - 0.0090
WKF	33	30	10 x 10	90.91	15 (0.0017)	0.0004 - 0.0017
WWM	34	28	5 x 10	82.35 (95.24)	10 (0.0015 - 0.0213)	0.0011 - 0.0213

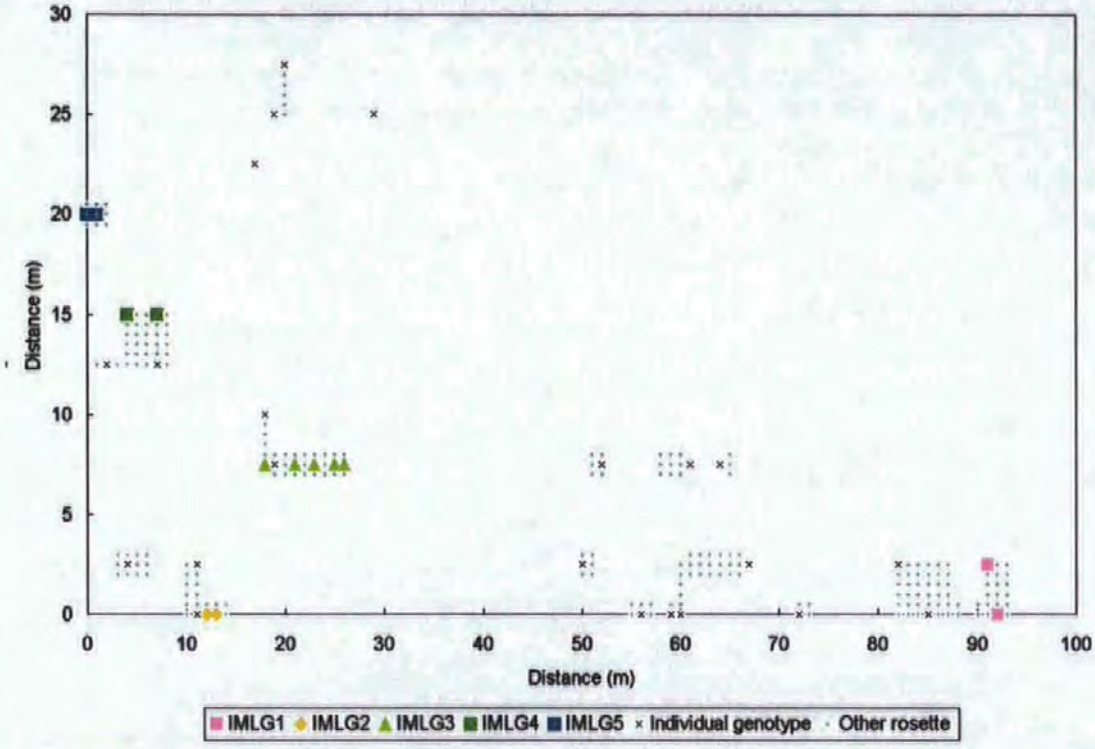


Figure 3.5 Schematic representation of the *Cirsium dissectum* population at Wicken Fen (EWF). Green plus signs indicate the size of each patch of *C. dissectum*. Thirty-five leaf samples were collected using a systematic sampling strategy and genotyped using 7 microsatellite loci. Black crosses represent plants with unique genotypes. Plants that shared the same multilocus genotype (IMLG) and therefore belong to the same clone are shown with the same symbol.

3.4 Discussion

3.4.1 Site characteristics and plant communities

The results of the soil analysis are similar to those observed by Ross (1999) and Goodwin (1995) for *C. dissectum* sites. Particularly characteristic were the low phosphorus levels; most sites had an ADAS Index of 0, whilst a smaller number reached 1, these values are typical for semi-natural, species-rich grasslands (Goodwin 1995; Tallowin & Smith 2001; Anon. 2005). The absence of *C. dissectum* from sites with higher phosphorus levels is likely to be the result of competition with species able to build up biomass more rapidly, such as *Molinia caerulea* (Jongejans *et al.* 2006a). The lowest pH recorded was 4.5;

ammonium toxicity possibly prevents *C. dissectum* from surviving in sites with a pH lower than this (de Graaf *et al.* 1998; Lucassen *et al.* 2002; Dorland *et al.* 2003). Potassium levels were variable (ADAS Index 0 to 4), with the mean being close to the range recommended for agricultural grassland production, suggesting that potassium levels are non-limiting in *C. dissectum* sites (Goodwin 1995). Clay-rich soils, such as the Culm grasslands, weather rapidly and release potassium-bearing minerals such as feldspars and micas. The potassium values for Giant's Causeway (IGC) were particularly high but this soil also appeared to have high clay content. Species-rich grasslands typically show a much wider range of potassium levels compared to phosphorus (Anon. 2005). Soil calcium levels were variable but often high, with some sites (Wicken Fen (EWF) and Bleach Lough (IBL)) having levels similar to those recorded for chalk grassland (Goodwin 1995). High calcium levels are a feature that has been recorded for *C. dissectum* sites previously (Wheeler & Shaw 1987; Hayati & Proctor 1991; Rodwell 1991; Ross 1999). Organic matter and total nitrogen varied between the low values observed in the dune slack sites such as Kenfig (WKF), to the high values recorded for peaty sites such as EWF. The differences between the dune slacks and other sites were also reflected in the plant communities within which *C. dissectum* was found. The species with which it was associated in the dune slacks and tall herb fen were very different from those in the rhos pastures and heaths. Species richness was high however throughout all of these communities, as expected for nutrient-poor grasslands.

It would have been useful to survey the plant communities for all 22 populations. This would have provided a comparison between the communities in Britain and Ireland and would have allowed plant community variables to be included in the examination of interactions covered in Chapter 4; unfortunately this was not possible due to time constraints.

3.4.2 Sexual reproduction

Variation in the proportion of plants flowering in different populations may be due to site nutrient levels and productivity as found by Jongejans *et al.* (submitted) for plants in the Netherlands. If this is the case, the increased flowering in some populations may increase the level of successful sexual reproduction in those sites (this will be investigated further in Chapter 4).

Seed production in *C. dissectum* appears to be lower than in some other *Cirsium* species. Up to 400 seeds per head, with six to 10 capitula per plant has been recorded in *C. vulgare* (Klinkhamer & de Jong 1993) and up to 160 seeds per head with 10 to 50 capitula in *C. eriophorum* (Tofts 1999). Seed dispersal also appears to be lower in *C. dissectum.* The maximum distance recorded was 21 m, whilst Tofts (1999) recorded 61 m for *C. eriophorum* and over 100 m for *C. vulgare. C. dissectum* therefore appears to have lower reproductive and colonisation capacity compared to these other *Cirsium* species.

Germination (9%) and seedling survival (27%) under standard conditions was low for *C. dissectum* and does not reflect the previously reported germination, and seedling survival potential of the species (Ross 1999). Seeds germinated in the same conditions collected the previous year had a mean germination of 41% and seedling survival of 50% and Ross (unpublished data) recorded even higher germination for stratified and non-stratified seeds germinated at similar temperatures to those used in this study (Chapter 1). There was no obvious cause for the low germination and seedling survival observed; the seeds were hard and well formed and were stored in the same conditions as the previous year. Fungal attack was prevented by fungicide solution but an unknown pathogen may have affected the seeds. Jongejans *et al.* (2006b) recorded significant variation between years in flower and seed production and it may be that there is also high natural temporal variation in germination and seedling survival.

Seed predation is an important factor in the population dynamics of British *Cirsium* species (Tofts 1999; Klinkhamer & de Jong 1993, Redfern 1968) and *C. dissectum* appears to be no exception to this. Redfern (1968) has recorded food-chains within the seed heads of *C. vulgare* and similar relationships seem to occur within *C. dissectum.* The chalicid wasps recorded from *C. dissectum* are likely to be ectoparasites on the Tephritid flies; Redfern (1968) recorded the chalicid *Habrocytus* sp. as a parasite on *Terrellia seratula* in *C. vulgare. C. dissectum* is also visited by two rare butterfly species, the marsh fritillary (*Eurodryas aurinia*) and swallowtail (*Papilio machaon britannicus*) and may be an important nectar source.

C. x *forsteri* was found in flower in 9% of populations and vegetative rosettes may have been present in others, Kenfig (WKF) in particular had *C.* x *forsteri* throughout the site. Hybridization could potentially have negative consequences for *C. dissectum* through genetic assimilation, outbreeding depression or hybrids competing for limited resources (Levin *et al.* 1996). This may be exacerbated by reductions in population size, caused by habitat destruction and fragmentation, as *C. dissectum* may be more likely to be assimilated by the more frequently occurring *C. palustre* (Levin *et al.* 1996). However, *C.* x *forsteri* has been recorded occurring with its parents throughout the range of *C. dissectum* but always at a low frequency and the loss of *C. dissectum* or *C. palustre* has never been described (Stace 1997; Preston *et al.* 2002). Infrequent hybridization events may actually be beneficial by increasing genetic diversity (Rieseberg 1991). This is an interesting area of further work and the different alleles observed in *C. palustre* and *C.* x *forsteri* suggests that microsatellite loci can be used to investigate hybridization in *Cirsium* species.

3.4.3 Asexual reproduction

Genotypic diversity (G/N) in *C. dissectum* was high at the 10 x 10 m spatial scale suggesting that, although seedling establishment is rarely seen, and makes up only a small

part of the species' population dynamics, successful sexual reproduction does occur. It is possible that somatic mutation within ramets causes some variation in multilocus genotypes but microsatellite markers are considered to be reliable for long-lived species, as somatic mutations do not significantly add to the rate of genomic mutations (Suvanto & Latva-Karjanmaa 2005). Two strategies of seedling recruitment are described from clonal species; in the former, seedling recruitment occurs early in the history of the population and then, as biomass builds up, seedling recruitment is prevented (initial seedling recruitment, ISR) (Eriksson 1997). If this is the case, then it is expected that the number of clones will gradually decrease over time, leaving a few larger genets, well adapted to local conditions (Watkinson & Powell 1993). The alternative is that seedling recruitment occurs throughout the history of the population (repeated seedling recruitment, RSR) (Eriksson 1997). The high proportion of distinguishable genotypes may suggest that repeated seedling recruitment occurs in most populations of *C. dissectum* but the examination of population dynamics does not support this (Jongejans *et al.* submitted). However, it is not uncommon for plants where sexual recruitment is rarely observed to maintain high levels of genotypic diversity (Eriksson *et al.* 1997). Soane & Watkinson (1979) modelled the dynamics of *Ranunculus repens* and found that, as the number of genets decreased over time, occasional seedling recruitment was sufficient to maintain high levels of genotypic diversity. An alternative explanation is that habitat heterogeneity maintains high genotypic diversity within populations, with different genets suited to different microhabitats (McLellan *et al.* 1997).

Braunton Burrows (EBB) had lower genotypic diversity compared to the other populations; this may be due to this population being founded by a small number of individuals and sexual recruitment being limited, maintaining a low number of genets. Alternatively, stronger selection pressure in the dune slack site may cause higher mortality

leaving a smaller number of well-adapted individuals; this will be discussed further in Chapter 5.

Examination of the clone map for Wicken Fen (EWF) shows that even in small populations, patches of rosettes are composed of many genets suggesting that rhizomes must intermingle as clonal propagation takes place. This mechanism should reduce levels of geitonogamous mating between capitula belonging to the same clone, making outcrossing more likely.

The maximum distance between identical ramets was 100 m; rhizome lengths in *C. dissectum* are variable from 0 to 40 cm; if the average is assumed to be 20 cm, then identical ramets separated by 100 m have potentially 500 intervening ramets. In common garden conditions, rosettes produce daughter ramets each year meaning that the clone in question could be 500 years old; in field conditions, clonal propagation may occur less frequently, making the clone older than this. This is a very approximate measure but similar calculations have been made for other clonal species. Parks & Werth (1993) recorded ramets spreading over a 1 km and being over 1000 years old in *Pteridium aquilinum*. Steinger *et al.* (1996) recorded a clone of *Carex curvula*, with 7000 tillers that was potentially 2000 years old. Clonal propagation allows individuals to live for a very long time and may improve survival chances by spreading the risk of stochastic events that lead to plant loss (Widén *et al.* 1994, McLellan *et al.* 1997).

3.4.4 Summary

C. dissectum is found in rhos pastures, wet heaths, fens and dune slacks and all of these habitats have low soil phosphorus and high plant species richness. In the British Isles, most rhos pastures and many heaths are cattle grazed, whilst tall herb fens and dune slack grasslands are typically mown.

C. dissectum is capable of sexual and asexual reproduction, with generally high levels of genotypic diversity at the 10 x 10 m spatial scale, suggesting that successful

sexual recruitment occurs. Nevertheless, seed production and dispersal is lower than observed in some other *Cirsium* species. Seedling recruitment rarely appears to occur in established vegetation and seed predation can be high.

Patches of *C. dissectum* rosettes are likely to be composed of more than one clone, with different genets intermingling. The majority of clones are 10 m in length or less but clones can spread for up to 100 m and potentially be over 500 years old.

Chapter 4

4 Genetic diversity within populations and interactions between population size, genetic diversity, fitness and habitat quality.

4.1 Introduction

Genetic analysis within populations can provide information on levels of genetic diversity and inbreeding (Lowe *et al.* 2004). Genetic diversity is important, as it allows species to adapt to changing environmental conditions. Populations with lower genetic diversity may have lower fitness and higher extinction risk (Barrett & Kohn 1991).

Inbreeding in plants can be part of the species breeding system and selfing species or geitonogamous clonal plants will have higher levels of inbreeding (Frankham *et al.* 2002; Charpentier 2002). Alternatively, inbreeding may be a consequence of changes in a population's environment, for example, a reduction in population size that reduces the number of mates available or loss of pollinators making cross-fertilisation less likely (Oostermeijer *et al.* 1998; Frankham *et al.* 2002). Inbreeding can cause inbreeding depression and this is often associated with increased seed abortion, low germination rates,

high seedling mortality, poor growth and poor flowering of offspring (Oostermeijer *et al.* 2003; Dudash & Fenster 2000).

Loss of genetic diversity and increases in levels of inbreeding are often observed when population sizes become smaller through habitat destruction and fragmentation. Relationships between population size, genetic diversity and fitness have been widely studied in plant species with significant, positive relationships often found between these three factors (Oostermeijer *et al.* 2003; Leimu *et al* 2006).

Habitat quality however, may also affect population size, genetic diversity and plant performance. Sexual recruitment for example, can be reduced or potentially permanently suppressed by environmental variables such as defoliation (mowing or grazing) (Schaal & Leverich 1996), canopy closure (Kudoh *et al.* 1999), climate (Eckert 1999) or an increase in site productivity (Colling *et al.* 2002; Endels *et al.* 2004). Such reductions in sexual recruitment often lead to a decrease in genetic diversity (Kudoh *et al.* 1999, Jacquemyn *et al.* 2005; 2006, Kleijn & Steinger 2002).

In natural populations, it is likely that population size, genetic diversity and habitat quality all interact to determine plant fitness and the survival of plant populations (**Figure 4.1**). In order to conserve species effectively, all of these factors need to be taken into account. Studies that attempt to look at interactions between these factors are therefore very important but are not frequent within the literature. Oostermeijer *et al.* (1998) demonstrated that habitat factors play an important role alongside population size and genetic diversity in the performance of the rare species *Gentiana pneumonanthe*. Vergeer *et al.* (2003) found that larger populations of *Succisa pratensis* had reduced inbreeding and greater fitness, and high soil ammonium had a negative effect on population size and fitness but did not affect genetic diversity. The author knows of no previous studies that have examined interactions between population size, genetic diversity, fitness and habitat quality in a clonal plant species.

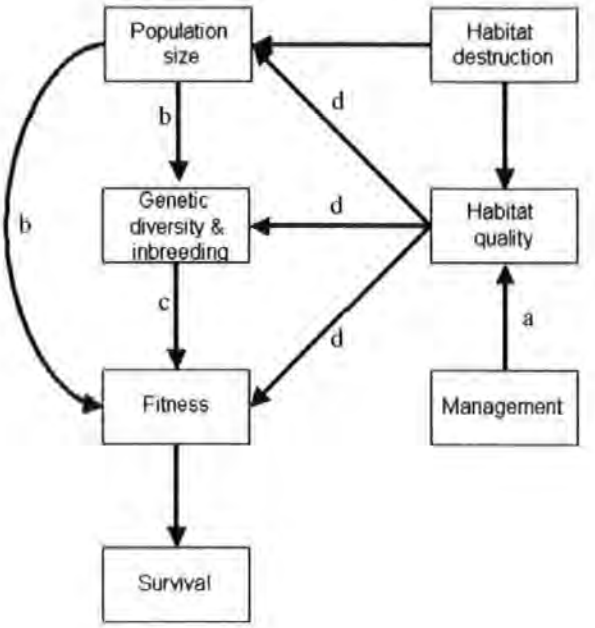


Figure 4.1 The theoretical relationships between habitat quality, population size, genetic diversity and fitness in plant species. (a) the effect of management on habitat quality; b) the effect of population size on genetic diversity and fitness; c) the effect of genetic diversity on fitness and d) the effect of habitat quality on population size, genetic diversity and fitness).

Chapter 3 has illustrated the range of variation in soil nutrients and vegetation structure found throughout *C. dissectum* sites. Sites are susceptible to modification through changes in site hydrology due to drainage and increases in site productivity through fertiliser use, aerial nitrogen deposition and natural succession (Fojt & Harding 1995; Jansen *et al.* 1996; Buck-Sorlin & Weeda 2000; van den Berg *et al.* 2005). The different management regimes described in Chapter 3, including mowing and different durations of grazing, are likely to change the structure and composition of the plant community.

Jongejans *et al.* (submitted) and Jongejans *et al.* (2006a) discovered that *C. dissectum* is a poor competitor relative to species such as *Molinia caerulea*, so increases in soil nutrients and site productivity are likely to decrease the probability of survival. Increases in nutrient levels also increase the proportion of rosettes that flower, although seedling recruitment is rarely observed and acts as a "bottleneck" for this species

(Jongejans *et al.* submitted). Nevertheless, the genotypic diversity described earlier (Chapter 3) shows that populations are typically composed of many individuals derived from sexual reproduction. Changes in vegetation structure and soil nutrients are therefore likely to have an effect on population size in *C. dissectum* and reductions in population size may lead to reductions in genetic diversity and fitness. Beyond this, changes in levels of flowering and seedling recruitment may have an impact on levels of sexual reproduction and this may also affect genetic diversity.

This chapter examines levels of genetic diversity and inbreeding within *C. dissectum* populations. It then examines the interactions between population size, genetic diversity, fitness and habitat quality. The measures of habitat quality concentrate on soil nutrients and vegetation structure and have been described in Chapter 3. Vegetation height is used as an indicator of site productivity and bare soil is measured as this may provide establishment gaps for seedlings. Phosphorus, nitrogen, potassium, organic matter, calcium and pH provide measures of soil fertility and composition. The specific objectives of this chapter are to:

1. Examine levels of genetic diversity and inbreeding within *C. dissectum* populations.
2. Investigate the interactions between genetic and genotypic diversity, population size, habitat quality and fitness by examining each of the components in **Figure 4.1**.
    a) The effect of management on habitat quality.
    b) The effect of population size on genetic diversity and fitness.
    c) The effect of genetic diversity on fitness.
    d) The effect of habitat quality on population size, genetic diversity and fitness.

3. Consider the implications of the results for the management of *C. dissectum* populations.

4.2 Methods and data analysis

4.2.1 Genetic diversity within populations

For each of the 22 populations previously described, 35 leaves were genotyped using seven microsatellite loci (see Chapter 2 for details). To determine genetic diversity GENETIX v.4.02 (Belkhir *et al.* 2001) was used to determine the proportion of polymorphic loci (P99), observed heterozygosity (H_o) and expected heterozygosity (H_e). Allelic richness (A) and the inbreeding coefficient (F_{IS}) were calculated using FSTAT v2.9.3.2 2002 (Goudet 2001). In order to test whether F_{IS} was significantly different from 0, a randomisation procedure was carried out in FSTAT, where alleles were randomised within populations with 154,000 permutations of the data being performed. If less than 5% of the randomised datasets resulted in F_{IS} that were more extreme than those observed then that population was considered not to be in Hardy-Weinberg equilibrium.

Each of these measures were calculated twice, first using all of the sampled plants (ramet level analysis) and secondly after the removal of identical multilocus genotypes (genet level analysis). This approach was taken as analyses containing all of the ramets could be biased due to individual samples not being independent (McLellan *et al.* 1997). However, removing identical multilocus genotypes ignores the fact that some clones may be more successful than others. This could lead to rare alleles being over-represented in the data and common alleles under-represented. Presenting both ramet and genet results helps to illustrate the effect of clonal reproduction on genetic diversity (McLellan *et al.* 1997).

4.2.2 Interactions between population size, genetic diversity, fitness and habitat quality.

Three measures of population size, three measures of fitness, nine habitat variables and four genetic variables were used to investigate the interactions between population size, genetic diversity, fitness and habitat quality in *C. dissectum* (**Table 4.1**).

Table 4.1 Summary of the variables measured and outline of the methods used (for detailed methods see Chapter 2).

	Variables	Method
Population size	Total number of rosettes	Population area x density of rosettes per m^2
	Number of flowering rosettes	Number of flowering rosettes counted in July
	Proportion of flowering rosettes (%)	Number of flowering and vegetative rosettes counted within 30 1 m^2 quadrats
Habitat	Vegetation height	Vegetation height and bare soil (%) were measured in 30 1 m^2 quadrats in July.
	Bare soil (%)	
	Phosphorus (mg kg^{-1})	5 soil samples taken per site, divided into 0-7cm and 7-14cm fractions, air-dried. pH electrometrically after mixing with distilled water. OM loss on ignition. P Olsen's method. Total Kjeldahl N. Ca and K extracted and determined using an atomic absorption spectrophotometer.
	Organic matter (OM) (%)	
	Total Nitrogen (%)	
	Potassium (mg kg^{-1})	
	Calcium (mg kg^{-1})	
	pH	
	Management	Owners and managers were asked to describe the management used on the site.
Genetic diversity	Proportion of polymorphic loci (P99)	35 leaves collected per population, phenol chloroform DNA extraction used. 7 microsatellite loci were amplified and the fragments visualised and alleles called using the CEQ 8000 Genetic Analysis System.
	Allelic richness (A)	
Inbreeding	Inbreeding coefficient (F_{IS})	
Genotypic diversity	G/N Proportion of distinguishable genotypes (10x10 m scale)	
Fitness measures	Mean seed number per capitulum	30 ripe seed heads collected per population. Number of hard seeds counted. Seed number adjusted for seed predation.
	Mean germination (%)	Plants were germinated and grown in standard glasshouse conditions.
	Mean seedling survival (%)	

For genetic diversity, the genet data were used, since the statistical methods adopted assume that each data point is independent (Tabachnick & Fidell 2007). For each of the population size, habitat quality and fitness measures, the mean was determined for each population. The mean number of seeds per capitulum was used as one of the measures of fitness but the number of hard seeds was affected by seed predation and levels of predation

varied widely between populations. Seed heads that were completely destroyed by seed predation were not included in the calculation of the population mean, as it was considered that this was a factor that was likely to vary considerably from year to year and did not reflect the fitness of the population.

Top and bottom soil fractions were analysed separately for each of the soil nutrients, so that any structuring of nutrients within the soil profile could be seen. In many cases, however, it was expected that the top and bottom fractions would be highly correlated. Similarly, the different soil nutrients measured were also expected to show some correlations. Multi-collinearity should be avoided when using multivariate statistics so bivariate correlations were determined between all soil variables (Tabachnick & Fidell 2007). If the top and bottom soil fraction had a correlation (r) of greater than 0.7, then the mean of the top and bottom was determined and used in subsequent analyses. In this way, means were used for all soil variables except phosphorus. Correlations were also high for organic matter and total Kjeldahl nitrogen ($r = 0.965$) and for calcium and pH ($r = 0.737$). Therefore nitrogen and calcium were removed from the multivariate analyses (multiple regression and structural equation modelling).

In order to obtain normality and homogeneity of variance within the data, the total number of rosettes, the number of flowering and the proportion of flowering rosettes, organic matter %, potassium mg kg^{-1}, and phosphorus (7-14 cm) mg kg^{-1} were log-transformed, the proportion of distinguishable genotypes was reflect-log transformed, whilst the proportion of polymorphic loci was arcsine-transformed prior to statistical analysis.

To examine the relationship between management type and habitat quality one-way ANOVAs with post-hoc Tukey tests were performed. Multiple regression analysis using forward, stepwise selection of variables was used to examine the effects of the other variables. The following groups of analyses were carried out:

a) the effect of population size on genetic diversity and fitness.

b) the effect of genetic diversity on fitness.

c) the effect of habitat quality on population size, genetic diversity and fitness.

The interactions between all of these factors were then investigated using structural equation modelling. A model was constructed that included all of the relationships shown using the multiple regression analyses, along with any correlations between the variables and the fit of this model to the data was tested. Maximum likelihood estimation was used to determine the standardised path coefficients; these are equivalent to standardised partial regression coefficients. Model fit was tested using the likelihood chi-squared value, which tests the null hypothesis that the covariance matrix implied by the model reproduces the observed covariance matrix and Bentler's comparative fit index (CFI) was calculated, where values greater than 0.9 indicate an acceptable fit between the model and the data (Byrne 2001; Iriondo *et al.* 2003; Grace 2006). AMOS 6 was used to design, estimate and test the model.

4.3 Results

4.3.1 Genetic diversity within populations

The mean level of allelic richness in *C. dissectum* was 2.60 for ramet and 2.49 for genet level analysis (**Table 4.2**). The lowest allelic richness was seen in Braunton Burrows (EBB) at 1.43 and the highest was Rans Wood (ERW) at 3.04 – 3.33. Values of allelic richness were consistently lower in genets compared to ramets, even if populations did not contain any identical multilocus genotypes. This is an artefact of the method used to calculate allelic richness in FSTAT. The observed number of alleles in a sample is dependent on the sample size so FSTAT uses a rarefaction index to create a measure of

allelic richness that is independent of sample size. The sample size is fixed as the smallest number of individuals genotyped for a locus in a sample. This means that comparisons of allelic richness within ramet or genet analyses are valid, but the difference in allelic richness between genets and ramets is not a reflection of the influence of clonal growth.

Most populations of *C. dissectum* were not in Hardy-Weinberg equilibrium, at the ramet level, 77% of populations had an excess of homozygotes and this rose to 82% when genets were analysed. Values of F_{IS} for ramets varied from –0.054 to 0.360, for genets 0.012 to 0.360 (**Table 4.3**).

Table 4.2 Genetic and genotypic diversity within *Cirsium dissectum*. (Allelic richness is the mean allelic richness averaged over seven microsatellite loci with the standard error (SE) in parentheses. P(99) is the proportion of polymorphic loci. For the calculation of genotypic diversity see Chapter 3).

	Ramets			Genets			Genotypic diversity
Population	N	Allelic richness (SE)	P(99)	G	Allelic richness (SE)	P(99)	G/N (10 x 10m)
EAC	35	2.71 (0.022)	1.00	35	2.65 (0.023)	1.00	100.00
EBB	35	1.43 (0.015)	0.43	17	1.43 (0.031)	0.43	64.71
EKS	34	2.49 (0.020)	1.00	32	2.34 (0.016)	1.00	94.12
EMA	35	2.27 (0.013)	1.00	34	2.19 (0.011)	1.00	97.14
EMO	32	2.96 (0.035)	1.00	28	2.82 (0.038)	1.00	87.50
EMS	34	2.84 (0.031)	1.00	34	2.73 (0.031)	1.00	100.00
ERW	34	3.33 (0.037)	1.00	33	3.04 (0.031)	1.00	97.06
EWF	35	2.14 (0.038)	0.57	27	2.10 (0.051)	0.57	100.00
IAR	35	2.93 (0.049)	0.86	32	2.69 (0.045)	0.86	91.43
IBL	35	3.08 (0.067)	0.86	35	2.89 (0.056)	0.86	100.00
IDL	34	2.81 (0.053)	0.86	33	2.69 (0.048)	0.86	97.06
IGC	35	2.69 (0.039)	0.86	28	2.43 (0.038)	0.86	80.00
ILB	33	2.86 (0.037)	1.00	33	2.79 (0.036)	1.00	100.00
ILG	35	2.43 (0.032)	0.86	33	2.40 (0.035)	0.86	94.29
ILL	35	2.98 (0.050)	1.00	35	2.84 (0.046)	1.00	100.00
ILT	35	2.90 (0.056)	0.86	35	2.66 (0.044)	0.86	100.00
IMA	34	2.29 (0.050)	0.71	31	2.25 (0.052)	0.71	91.18
IME	35	2.67 (0.046)	0.86	25	2.59 (0.059)	0.86	94.44
WCG	34	2.99 (0.047)	0.86	34	2.80 (0.039)	0.86	100.00
WDB	33	2.13 (0.020)	0.86	31	2.05 (0.018)	0.86	93.94
WKF	33	2.54 (0.029)	0.86	30	2.44 (0.030)	0.86	90.91
WWM	34	1.86 (0.011)	0.86	28	1.85 (0.016)	0.86	95.24
Mean		**2.60**	**0.87**		**2.49**	**0.87**	**94.05**
SE		**0.020**	**0.01**		**0.018**	**0.01**	**1.76**

Table 4.3 Observed (H_o) and expected (H_e) heterozygosity and inbreeding coefficient (F_{IS}) in *Cirsium dissectum*. (For observed and expected heterozygosity the mean is given for the seven microsatellite loci with the standard error (SE) in parentheses. *P*-values for F_{IS} were obtained in a randomisation test of $F_{IS} = 0$ based on 154,000 permutations of the data in which alleles were randomised within populations. *** $P < 0.001$, * < 0.05).

	Ramets			Genets		
Population	H_o **(SE)**	H_e **(SE)**	F_{IS}	H_o **(SE)**	H_e **(SE)**	F_{IS}
EAC	0.388 (0.007)	0.485 (0.006)	0.202***	0.388 (0.007)	0.485 (0.006)	0.202***
EBB	0.224 (0.009)	0.213 (0.008)	-0.054	0.160 (0.013)	0.213 (0.016)	0.258
EKS	0.319 (0.005)	0.427 (0.003)	0.258***	0.316 (0.005)	0.429 (0.004)	0.266***
EMA	0.326 (0.005)	0.408 (0.005)	0.204***	0.328 (0.005)	0.411 (0.005)	0.205***
EMO	0.380 (0.008)	0.493 (0.006)	0.232***	0.383 (0.009)	0.498 (0.007)	0.234***
EMS	0.386 (0.006)	0.467 (0.006)	0.176***	0.386 (0.006)	0.467 (0.006)	0.176***
ERW	0.449 (0.007)	0.523 (0.005)	0.144***	0.439 (0.007)	0.518 (0.005)	0.154***
EWF	0.285 (0.008)	0.317 (0.009)	0.101	0.262 (0.010)	0.331 (0.013)	0.210***
IAR	0.341 (0.008)	0.348 (0.008)	0.020	0.348 (0.009)	0.352 (0.009)	0.012
IBL	0.360 (0.006)	0.473 (0.007)	0.241***	0.360 (0.006)	0.473 (0.007)	0.241***
IDL	0.352 (0.008)	0.473 (0.008)	0.260***	0.356 (0.009)	0.474 (0.008)	0.252***
IGC	0.276 (0.007)	0.399 (0.006)	0.312***	0.275 (0.008)	0.399 (0.007)	0.316***
ILB	0.385 (0.008)	0.463 (0.007)	0.171***	0.385 (0.008)	0.463 (0.007)	0.171***
ILG	0.293 (0.006)	0.343 (0.007)	0.148***	0.289 (0.007)	0.347 (0.007)	0.170***
ILL	0.409 (0.008)	0.480 (0.007)	0.151***	0.409 (0.008)	0.480 (0.007)	0.151***
ILT	0.281 (0.007)	0.437 (0.006)	0.360***	0.281 (0.007)	0.437 (0.006)	0.360***
IMA	0.270 (0.009)	0.342 (0.008)	0.214***	0.288 (0.010)	0.343 (0.009)	0.165*
IME	0.341 (0.008)	0.423 (0.006)	0.195***	0.354 (0.011)	0.461 (0.009)	0.236***
WCG	0.390 (0.008)	0.464 (0.007)	0.163***	0.390 (0.008)	0.464 (0.007)	0.163***
WDB	0.376 (0.009)	0.374 (0.006)	-0.007	0.367 (0.009)	0.376 (0.006)	0.023
WKF	0.424 (0.007)	0.451 (0.007)	0.060	0.423 (0.008)	0.457 (0.007)	0.075
WWM	0.270 (0.006)	0.334 (0.005)	0.194***	0.269 (0.008)	0.336 (0.007)	0.203*

4.3.2 Relationships between site management and habitat variables

Sites with different management showed variations in the habitat variables measured (**Table 4.4**). Sites that were subject to mowing had less potassium, higher calcium and higher pH compared to those that were grazed. As the level of grazing intensity increased from none to summer to continuous, the amount of bare soil increased and vegetation height and organic matter decreased. There were also marginally significant trends showing decreased phosphorus (7-14 cm) and nitrogen.

Table 4.4 Mean and standard error (SE) in parentheses for vegetation and soil nutrient values under different site management regimes: none, summer grazing, continuous grazing or cutting. (Results of a one-way ANOVA are shown (mean square, F and *P*) and *post-hoc* Tukey tests. Sites that do not share a letter are significantly different).

	None	Summer	Continuous	Cut	Mean Square	F	*P*
Vegn. height (mm)	597.6 (1.3) a	411.1 (24.6) ab	192.0 (40.0) b	407.7 (148.1) ab	82283.74	3.64	0.039
Bare soil (%)	0.0 (0.0) a	4.6 (1.8) a	19.2 (0.8) b	6.5 (2.6) a	244.67	12.48	0.000
Phosphorus 0-7cm (mg kg $^{-1}$)	6.4 (4.2)	3.9 (1.6)	4.9 (2.7)	2.8 (2.0)	0.56	0.32	0.807
Phosphorus 7-14cm (mg kg $^{-1}$)	3.1 (2.0)	1.6 (0.3)	0.8 (0.2)	1.0 (0.2)	0.05	2.61	0.093
Organic matter (%)	83.1 (3.7) a	27.0 (6.9) ab	12.6 (1.4) b	27.5 (18.2) ab	0.34	3.94	0.031
Nitrogen (%)	1.8 (0.1)	0.6 (0.2)	0.3 (0.1)	0.7 (0.6)	0.36	2.93	0.070
Potassium (mg kg $^{-1}$)	147.7 (53.4) a	142.4 (17.7) a	74.6 (16.3) ab	42.5 (12.7) b	0.32	8.44	0.002
Calcium (mg kg $^{-1}$)	3193 (1392) ab	2220 (4119) ab	792 (451) a	6156 (2944) b	0.88	4.22	0.025
pH	4.8 (0.2) ab	4.9 (0.1) a	4.9 (0.2) a	5.7 (0.2) b	0.74	4.62	0.019

4.3.3 Interactions between population size, genetic diversity, fitness and habitat quality.

The structural equation model (**Figure 4.2**) containing all of the relationships found in the multiple regression analyses (**Table 4.5** to **Table 4.7**) showed a good fit between the model and the data; this was indicated by a chi-squared of 42.13; df 46; *P* 0.635 and a CFI of 1. Population size (total number of rosettes) had a significant positive relationship with genetic diversity (proportion of polymorphic loci); another measure of genetic diversity

(allelic richness) had a significant positive relationship with plant fitness (seedling survival).

Of the seven habitat variables included in the analysis, four showed relationships with population size, genetic diversity and fitness. Three of these variables were also significantly correlated with each other; vegetation height was positively correlated with phosphorus levels and bare soil was negatively correlated with vegetation height and with phosphorus. Greater numbers of *C. dissectum* rosettes were associated with sites with shorter vegetation and lower pH; sites with shorter vegetation, however, had proportionally less rosettes in flower and the more bare soil, the fewer the total number of flowering rosettes. Sites with tall vegetation and little bare soil therefore have fewer rosettes but those rosettes are more likely to be in flower.

There were relationships between habitat quality and genetic diversity; sites with more bare soil had *C. dissectum* populations with greater allelic richness. Sites with more phosphorus (7–14 cm) also had greater allelic richness and reduced levels of inbreeding. Finally, habitat quality was related to fitness as sites with more phosphorus showed greater germination of *C. dissectum* seeds under standard conditions.

Table 4.5 Relationships between population size (total number of rosettes, number of flowering rosettes and proportion of flowering rosettes) genetic and genotypic diversity (proportion of polymorphic loci, allelic richness, inbreeding coefficient, proportion of distinguishable genotypes) and fitness (seed number, germination, seedling survival). (Multiple regression analysis with stepwise selection of variables was performed for each dependent variable using the independent variables shown in the table. R^2 is shown for each test, the * symbol next to the R^2 value indicates the level of significance of the test, *** = *P*-value of < 0.001. The β, t and *P* values are shown for each significant independent variable, nr = no relationships found).

Dependent variable for each analysis	R^2 in multiple regression	Independent variables		
		Total number of rosettes	Number of flowering rosettes	Proportion of flowering rosettes
Prop. of polymorphic loci (P99)	0.323***	β 0.568 t 3.088 *P* 0.006	nr	
Allelic richness (A)	nr			
Inbreeding coefficient (F_{IS})	nr			
Prop. of distinguishable genotypes (G/N)	nr			
Mean seed number	nr			
Mean % germination	nr			
Mean % seedling survival	nr			

Table 4.6 Relationships between genetic and genotypic diversity (proportion of polymorphic loci, allelic richness, inbreeding coefficient, proportion of distinguishable genotypes) and fitness (seed number, germination, seedling survival). (Multiple regression analysis with stepwise selection of variables was performed for each dependent variable using the independent variables shown in the table. R^2 is shown for each test, the * symbol next to the R^2 value indicates the level of significance of the test, * = *P*-value of < 0.05. The β, t and *P* values are shown for each significant independent variable, nr = no relationships found).

Dependent variable for each analysis	R^2 in multiple regression	Independent variables			
		Prop. of polymorphic loci (P99)	Allelic richness (A)	Inbreeding coefficient (F_{IS})	Prop. of distinguishable genotypes (G/N)
Mean % seedling survival	0.270*	nr	β 0.519 t 2.431 *P* 0.027	nr	
Mean seed number	nr				
Mean % germination	nr				

Table 4.7 Relationships between habitat (vegetation height, bare soil, phosphorus (0-7 cm and 7-14 cm), organic matter, potassium, pH), population size (total number of rosettes, number of flowering rosettes and proportion of flowering rosettes), genetic and genotypic diversity (proportion of polymorphic loci, allelic richness, inbreeding coefficient, proportion of distinguishable genotypes) and fitness (seed number, germination, seedling survival). (Multiple regression analysis with stepwise selection of variables was performed for each dependent variable using the independent variables shown in the table. R^2 is shown for each test, the * symbol next to the R^2 value indicates the level of significance of the test, * = *P*-value of < 0.05, ** = *P*-value of < 0.01. The β, t and *P* values are shown for each significant independent variable, nr = no relationships found. The relationship between proportion of flowering rosettes and vegetation height had an outlier with a standardised residual of 3.11 with the outlier removed $R^2 = 0.544$, β = 0.753, t = 4.99, *P* = < 0.001).

Dependent variable for each analysis	R^2 in multiple regression	Independent variables						
		Vegn. height	Bare soil	P 0-7 cm	P 7-14 cm	OM	K	pH
Total number of rosettes	0.497**	β -0.637 t -3.862 *P* 0.001	nr					β -0.423 t -2.566 *P* 0.019
Number of flowering rosettes	0.269*	nr	β -0.519 t -2.716 *P* 0.013	nr				
Prop. of flowering rosettes	0.300**	β 0.548 t 2.930 *P* 0.008	nr					
Prop. of polymorphic loci (P99)	nr							
Allelic richness (A)	0.546**	nr	β 0.824 t 4.666 *P* <0.001	nr	β 0.560 t 3.173 *P* 0.005	nr		
Inbreeding coefficient (F_{IS})	0.287**	nr			β -0.536 t -2.837 *P* 0.010	nr		
Prop. of distinguishable genotypes (G/N)	nr							
Mean seed number	nr							
Mean % germination	0.582**	nr			β 0.763 t 5.272 *P* <0.001	nr		
Mean % seedling survival	nr							

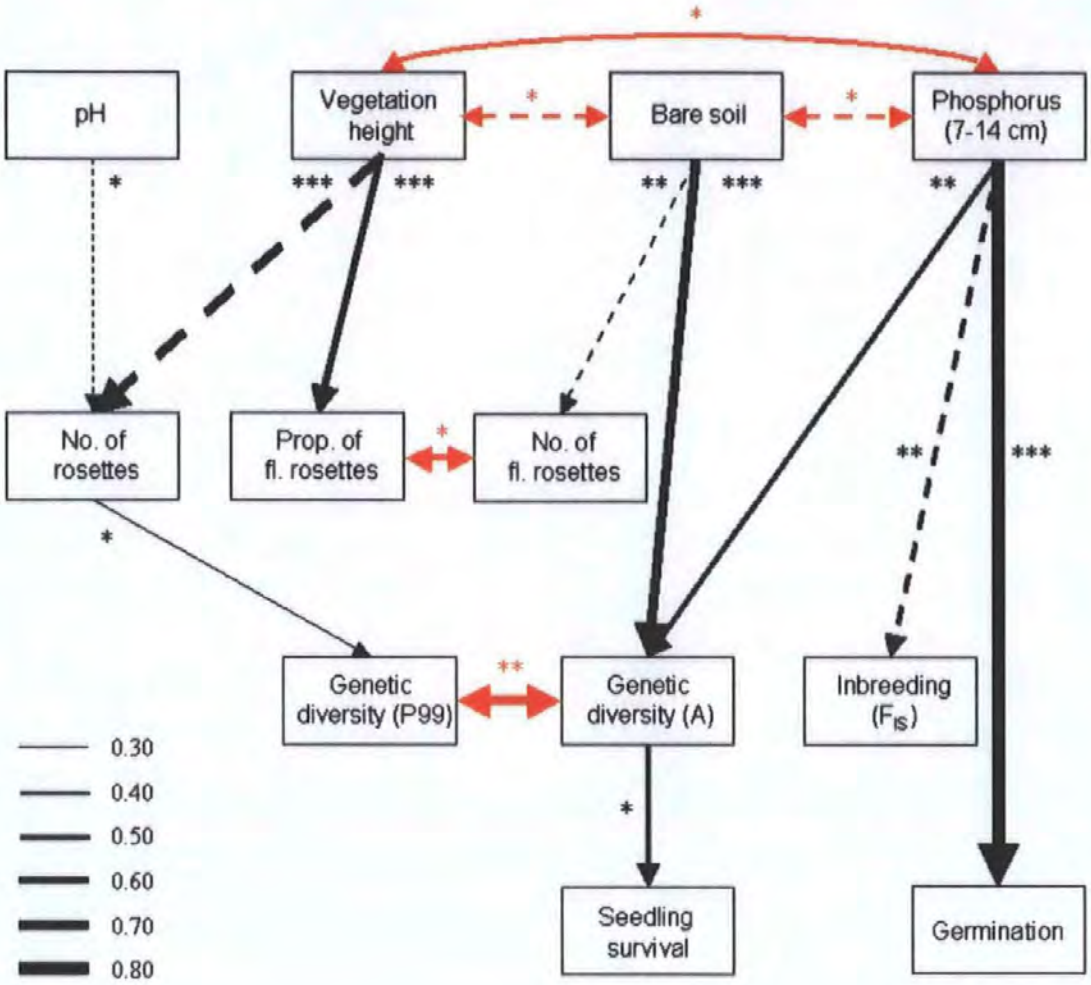


Figure 4.2 A structural equation model illustrating the relationships between population size, genetic diversity, fitness and habitat quality in *Cirsium dissectum* constructed using the relationships determined during multiple regression analyses. (Black single headed arrows indicate causal relationships between variables and red double-headed arrows show correlations between unmeasured residual variance between variables. The width of each arrow is proportional to the standardised path coefficient, with solid lines indicating positive relationships and dashed lines negative. Asterisks indicate significant relationships: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. The chi-squared statistic is 42.128, df = 46, $P = 0.635$, CFI = 1.00, indicating a good fit between the model and the data).

4.4 Discussion

4.4.1 Genetic diversity within populations

The positive inbreeding coefficients (F_{IS}) seen in *C. dissectum* suggests that a certain amount of inbreeding occurs, either through mating between close relatives or selfing

(Lowe *et al.* 2004). The fact that most populations show an excess of homozygotes and that inbreeding levels do not relate to any of the measures of population size suggests that the level of inbreeding at least partially reflects the mating system of *C. dissectum.* Geitonogamous mating in clonal plants will increase levels of inbreeding (Charpentier 2002) but the intermingling of genets described in Chapter 3 would be expected to reduce matings between different capitula belonging to the same clone. It is likely that *C. dissectum* has a mixed mating system with both outcrossing and selfing occurring. This is investigated further in Chapter 5.

4.4.2 Relationships between site management and habitat variables

There were significant relationships between site management, vegetation structure and soil nutrients. The cut and grazed sites varied in many ways. The cut sites included the dune slacks and tall herb fen whilst the grazed sites included rhos pastures and heaths. As the cut versus grazed sites represent very different habitats, the differences observed are not simply due to management. The grazed sites however represent more comparable habitats, so the differences observed here are likely to be at least partly due to the influence of grazing levels. The results suggest that increased grazing duration is likely to lead to shorter vegetation, more bare ground and some reductions in soil fertility.

4.4.3 Interactions between population size, genetic diversity, fitness and habitat quality

Population size, genetic diversity, habitat quality and fitness are correlated in a number of ways and the long-term survival of populations will be influenced by interactions between these. The results show significant, positive relationships between population size (measured as the total number of rosettes), genetic diversity and fitness (seedling survival). Lower genetic diversity in small populations may be due to the loss of rare alleles through genetic drift leading eventually to the fixation of loci (Frankham *et al.* 2002). This loss of

genetic diversity can reduce the ability of a species to adapt to changing environmental conditions. This may explain the relationship observed between allelic richness and seedling survival in standard conditions. Growing plants in glasshouse conditions, where all of their requirements are provided, may not be expected to place strong selection pressures on the plants. However, seedling survival was relatively low (Chapter 3), possibly due to an unidentified pathogen. If this was the case, then the plants may have been under selection pressure and some of those that came from populations with higher allelic richness were better able to survive. Alternatively, maternal effects could explain the relationship between allelic richness and seedling survival. Mother plants found in more suitable environments may have greater allelic richness and these environments may also improve the survival chances of the seedlings.

Habitat variables were related to population size, genetic diversity and fitness in *C. dissectum*. Heavily grazed sites with short vegetation and abundant bare soil tended to have more *C. dissectum* rosettes but fewer of those rosettes flowered. This feature was immediately apparent during site visits, with some populations with short swards and bare soil having no rosettes in flower at all, whilst others had tall vegetation and abundant flowers. For example, Baddesley Common (EBC) and Knowstone Moor (EKS) (Appendix A) were both M24c grasslands that differed in grazing intensity. EKS had abundant flowering rosettes, whilst no flowering rosettes were seen in EBC during visits over two summers.

Ross (1999) showed that experimentally defoliated *C. dissectum* plants produced more ramets than un-defoliated individuals. The larger number of rosettes in sites with short vegetation may therefore be due to increased levels of clonal growth, due to greater grazing intensity. Grazing may also reduce the proportion of rosettes that flower. Bullock *et al.* (1994) showed that winter grazing of sites containing *Cirsium vulgare* increased the survival of smaller rosettes in the population and thus decreased the proportion of rosettes

flowering. The reduced flowering in sites with short vegetation may also reflect lower productivity as Jongejans *et al.* (submitted) found that more productive sites (estimated as the biomass of clipped vegetation) had a greater proportion of flowering rosettes in grasslands in the Netherlands and that adding fertiliser to *C. dissectum* in experimental conditions increased flowering.

This study shows a possible influence of habitat variables on genetic diversity in *C. dissectum* with phosphorus (7–14 cm) and bare soil showing significant positive relationships with levels of allelic richness. Both these factors may lead to increased genetic diversity through increasing levels of successful sexual reproduction. Bare soil is likely to be especially important as it provides establishment gaps for seedlings. Seedling recruitment is reported to act as a "bottleneck" in the population dynamics of this species (Kay & John 1994; Jongejans *et al.* 2006b; Jongejans *et al.* submitted) and establishment of seedlings is promoted by disturbance, such as sod-cutting, that creates bare soil (Jongejans *et al.* 2006b; Jongejans *et al.* submitted; Isselstein *et al.* 2002). This suggests that sites that do not have bare soil will show a loss of genetic diversity over time, as long-lived clones gradually die and are not replaced with new sexual recruits. It is generally considered that levels of sexual recruitment in clonal plants do not have to be high in order to maintain genetic diversity (Watkinson and Powell 1993; Stehlik and Holdregger 2000) but if sexual recruitment is very low or non-existent, then reductions in genetic diversity are to be expected, and other studies have shown reductions in genetic diversity in clonal plants where sexual recruitment is suppressed (Kudoh *et al.* 1999; Jacquemyn *et al.* 2005; 2006; Kleijn and Steinger 2002).

The relationship between phosphorus and genetic diversity may be due to a number of mechanisms. Jongejans *et al.* (submitted) has shown that adding nutrients to *C. dissectum* increased flowering and this could subsequently relate to increased genetic diversity if greater flowering increases sexual reproduction. The reduction in inbreeding in

sites with higher phosphorus fits this hypothesis, as more flowering will increase the number of mates available and may promote greater pollination (Oostermeijer *et al.* 1998; Frankham *et al.* 2002). However, the structural equation model shows no relationship between phosphorus levels and increased flowering. In this study, the number of flowering rosettes was only counted for one year, whereas it would be preferable to count the number of flowers produced over a number of years to account for annual variation. Phosphorus levels may thus be an indicator of greater flowering potential over time. Other hypotheses include the possibility that phosphorus may promote sexual reproduction by increasing the survival of seedlings, and sites with more phosphorus did have seeds that germinated better under standard conditions, so this may increase the chances of seedling survival in field sites.

A further hypothesis is that phosphorus and bare soil do not relate to increased genetic diversity just through the promotion of sexual reproduction, but also allow the maintenance of genetic diversity, by increasing the survival chances of clones. This mechanism is unlikely for phosphorus, as *C. dissectum* is likely to be out-competed as nutrient levels increase. Bare soil however, may be important for the establishment of clonal offspring ensuring the long-term survival of clones, and the greater number of rosettes in sites with more bare soil supports this suggestion. Bare soil may therefore increase genetic diversity through increasing successful sexual and clonal reproduction; sexual reproduction introduces new genotypes into the population, whilst clonal propagation ensures their long-term survival.

Ultimately, the approach taken here, where a number of variables are measured in natural populations and then the relationships between them investigated, has the disadvantage that definite mechanisms cannot be provided for the relationships found. The approach is valuable however, in that it provides hypotheses that can subsequently be tested experimentally. It is also valuable in that, by taking into consideration some of the

interacting factors, it is possible to make management recommendations based on the results.

4.4.4 Conclusions and conservation implications

Small populations of *C. dissectum* have less genetic diversity and reduced genetic diversity affects fitness and subsequently the survival potential of populations. The protection of large existing populations of *C. dissectum* and the expansion of smaller populations is therefore an important recommendation for the conservation of this species. Beyond this, site management and habitat quality are related to survival probabilities. Unmanaged sites with tall vegetation and no bare soil, are likely to have abundant flowering of *C. dissectum* rosettes. Lack of bare soil, however, prevents the establishment of seedlings and this suppression of sexual recruitment causes a loss of genetic diversity that may decrease plant fitness and long-term population survival probabilities. Furthermore, *C. dissectum* is unable to build up biomass as rapidly as co-occurring species such as *Molinia caerulea,* so in populations with high nutrient levels, *C. dissectum* will eventually become out-competed (Jongejans *et al.* submitted).

Conversely, sites that are continually grazed have shorter vegetation, more bare soil and some depletion in nutrients. These sites have more *C. dissectum* rosettes, possibly through increases in clonal growth, but flowering is reduced. If flowering is completely suppressed, then sexual reproduction will not be able to occur and genetic diversity will eventually be lost. Similarly, if phosphorus levels become too low, this will lead to reductions in genetic diversity, possibly through reduced flowering or seedling survival.

Site management therefore needs to maintain habitat heterogeneity, so that some flowering can occur, levels of phosphorus are not depleted, and areas of bare soil are maintained to allow for successful recruitment of seedlings and clonal offspring. It is important to note, however, that phosphorus levels are very low in *C. dissectum* sites (the

mean is only 1.4 mg kg^{-1} at 7 to 14 cm) and if phosphorus levels become too high, then *C. dissectum* is likely to be out-competed by other plant species.

This considers populations as isolated entities but it is possible that productive sites with abundant flowering act as seed sources for other sites where bare soil is available. This mechanism seems to occur in the Netherlands, where topsoil is removed from restoration sites established next to existing populations (Jongejans 2004). Soons & Heil (2002) investigated the effect of site productivity on the ability of *C. dissectum* to colonise new areas of the Netherlands. More productive sites had higher seed production and percentage germination under standard conditions but seed dispersal ability decreased, reducing the chance of longer distance dispersal. Soons *et al.* (2005) related seed dispersal ability to the availability of suitable habitat within an area of the Netherlands in order to investigate habitat connectivity. The remaining grasslands containing *C. dissectum* were found to be practically isolated from each other in terms of dispersal, with the regional survival of the species being completely dependent on a few large populations in nature reserves. Metapopulation dynamics, therefore, does not appear to be a major factor in the survival of *C. dissectum* and this further emphasises the importance of ensuring that the whole life-cycle is able to occur within a single population.

Chapter 5

5 Genetic differentiation in *Cirsium dissectum* and the effect of inbreeding and outbreeding.

5.1 Introduction

Knowledge of the levels and distribution of genetic variation within a species is a prerequisite in the development of effective conservation plans (Hamrick *et al.* 1991). Analysis of the genetic structure within a plant species can illustrate how genetic variation is partitioned within and between populations. If populations are highly differentiated, then more populations will need to be conserved in order to capture the same proportion of variation as in a less differentiated species (Hamrick *et al.* 1991).

The pattern of genetic variation between populations provides information on current and historical levels of gene flow, as differentiation is caused when gene flow is reduced or prevented between populations. Within the British Isles, most plant species retreated to warmer refugia during the Pleistocene glaciation and current patterns of genetic differentiation can reflect the species' post-glacial re-colonization route (Hewitt

2000; Chauvet *et al.* 2004). Current processes that affect differentiation include habitat destruction and fragmentation; geographically isolated populations may gradually become differentiated through genetic drift and this tends to be more pronounced in smaller populations (Frankham *et al.* 2002). Strong selection pressures can also encourage divergence (Endler 1986). A pattern that is frequently observed is isolation by distance. This is a consequence of the limited dispersal capacity of plants and means that, as geographic distance increases, gene flow becomes less likely leading to a correlation between genetic and geographic distance (Bockelmann *et al.* 2003).

Analysis of plant genetic structure can highlight populations with a different genetic makeup and this can be used to determine populations that are important to conserve (Hamrick *et al.* 1991). Knowledge of genetic structure is also important if plants are to be translocated, either to rescue threatened populations or to re-create habitat that has been lost. Habitat re-creation is seen as a method for restoring connectivity between small and isolated habitat fragments (Gilbert and Anderson 1998). This introduces the question of where individuals for the new habitat should be sourced. Many authorities state that plants used in re-creation projects should be of local provenance (Gilbert and Anderson 1998). Others suggest that it is more important to maximise levels of genetic diversity so that populations have sufficient genetic variation to adapt to changing environmental conditions (Booy *et al.* 2000).

Local provenance is often considered to be important, since plants that are introduced from more distant locations may not be able to survive as well as local ecotypes (Jones *et al.* 2001). If translocated plants from different populations hybridize, this may lead to lower fitness due to outbreeding depression. This can arise if populations are highly adapted to their environment, as mixing their gene pools may then lead to offspring that are adapted to neither parental environment: this has been named the "ecological mechanism" (Luijten *et al.* 2002). Alternatively, it is suggested that outbreeding depression can be

caused by the disruption of co-adapted gene complexes or deleterious interactions when heterozygotes or homozygotes interact: these are called "genetic mechanisms" (Luijten *et al.* 2002; Edmands 2007).

The problem with local provenance is that, if local populations are small, the level of genetic diversity may be low, meaning that the reintroduced population will have less ability to adapt to changing environmental conditions (Barrett and Kohn 1991). Small populations may also be subjected to greater inbreeding and this may lead to inbreeding depression that will also lower offspring fitness (Edmands 2007). Even if local populations are large and genetically diverse, the seed collected from one local population will only represent a subset of the total variation found within that population (Smulders *et al.* 2000). Reintroduced populations composed of a small number of individuals may again suffer from low genetic diversity and inbreeding.

The relative effects of inbreeding and outbreeding therefore need to be considered together when choosing source populations for reintroductions. Mating system is likely to influence the susceptibility of a species to inbreeding and outbreeding depression, with inbreeding depression expected to be greater in outcrossing than in selfing populations, as the latter are more likely to have purged deleterious alleles (Fenster & Dudash 1994). In contrast, species with a high tendency for selfing may be more susceptible to outbreeding depression (Fenster & Dudash 1994). Levels of outbreeding depression will depend on the degree of differentiation between populations, greater genetic divergence may lead to a greater reduction in hybrid fitness (Edmands 2007). An examination of genetic structure can reveal levels of differentiation but cannot indicate at what point outbreeding depression may occur as the level of divergence that causes problems is highly variable between different species (Edmands 2007).

Genetic markers such as AFLP and microsatellites are widely used to determine patterns of differentiation (Lowe *et al.* 2004) but their use has been criticised, as neutral

markers may not necessarily be suitable to quantify selectively important variation (Booy *et al.* 2000; Ouborg *et al.* 2006). It is valuable to examine morphological differences as well as variation measured using neutral genetic markers (Ouborg *et al.* 1991; 2006).

Earlier (Chapter 1) the conservation status of *C. dissectum* was described and this highlighted the fact that it is endangered in Germany and the Netherlands and has declined in all of the other countries within which it is found. It is a key species of Cirsio-Molinietum grassland, a habitat that has declined substantially throughout Europe. Previous research has been reported in the Netherlands (Berendse *et al* 1992; Jansen & Roelofs 1996; Jansen *et al* 1996; Beltman *et al* 2001) and the UK (Tallowin & Smith 2001) describing the best methods for restoring Cirsio-Molinietum fen meadows. The UK Biodiversity Action Plan lists purple moor grass and rush pasture as a priority habitat with plans to re-create this habitat on land adjacent to or nearby existing sites (HMSO 1995). Information on which populations should be considered as conservation priorities and the best source of plant material to use in any habitat restoration projects is therefore of conservation importance.

As described in Chapter 1, a limited amount of previous research has been reported examining genetic structure within *C. dissectum.* Kay and John (1994) examined levels of genetic diversity within and between populations of *C. dissectum,* predominantly in Wales, using allozyme markers. Most of the populations studied were monomorphic and identical and this very low level of polymorphism limited the conclusions that could be drawn from their results. Smulders *et al.* (2000) used AFLP to investigate genetic diversity between source and reintroduced populations of *C. dissectum* in the Netherlands. Five source populations were sampled, varying from a few kilometres to a maximum of 200 km from the reintroduction site. Two thousand seeds from each source population were introduced into experimental plots at the restoration site and these were genotyped using AFLP the following year. Source populations showed small but significant genetic differences from

each other. The first generation of reintroduced plants showed less genetic variation than their source populations and were also genetically differentiated from them. Calculations showed that reintroduction from more than one source population introduced significantly more genetic variation, and Smulders *et al.* (2000) suggested that this might be the best strategy for plants in the Netherlands. Only a small number of populations were examined however and outbreeding depression was not considered.

This chapter investigates genetic differentiation in *C. dissectum* and the effect of inbreeding and outbreeding on early fitness traits by means of three investigations: first genetic structure and differentiation was examined for 22 populations of *C. dissectum* throughout the British Isles, using microsatellite genetic markers. Secondly, morphological traits were measured on *C. dissectum* plants from different populations grown under standard conditions to examine the relationship between genetic differentiation measured using neutral genetic markers and morphological variation. Thirdly, a simple pot experiment was conducted to investigate the effect of different intra- and inter-population crosses on seed production and germination. Specifically, this chapter has the following objectives.

a) Examine how genetic variation is partitioned within *C. dissectum.*

b) Describe genetic differentiation between populations and test whether genetic variation can be explained by isolation by distance.

c) Investigate whether plants that are genetically different, as measured using microsatellite markers, have morphological differences when plants are grown in standard conditions.

d) Conduct intra- and inter-population crosses to test whether *C. dissectum* is self-compatible and the effects of inbreeding and outbreeding on seed production and germination.

e) Consider the implications of the results for the conservation of populations and the source of plant material for habitat re-creation projects.

5.2 Methods

Detailed methods are described in Chapter 2 and a brief summary provided here. To examine genetic structure and differentiation, 35 leaves were collected from each of 22 populations of *C. dissectum* throughout the British Isles. These were genotyped using seven microsatellite loci.

Morphological traits were measured on plants grown from seed collected from the wild over two years (2003 and 2004) and grown in standard conditions in a net-tunnel. Fourteen vegetative characters were measured on one-year-old plants (see Chapter 2, **Table 2.3**). Plants in 2003 came from nine populations in England and Wales, whilst in 2004 plants came from 14 populations in England, Wales and Ireland (see Chapter 2, Table 2.1).

To investigate inbreeding and outbreeding, the following crosses were carried out on flowering plants grown in standard conditions.

1. Selfed within a capitula (19 plants).
2. Within population (14 crosses).
3. Populations up to 30 km apart (9 crosses).
4. Populations 35 – 100 km apart (15 crosses).
5. Populations 110 – 450 km apart (12 crosses).

Reciprocal crosses were carried out by hand using a paintbrush to transfer pollen and plants were isolated using muslin bags. Crosses were carried out each day, from the

appearance of the first flower, to the point when all flowering within the capitulum was complete. Ripe seed heads were dissected, the number of hard and shrivelled seeds counted and germination measured, using the methods described in section 2.5.

5.2.1 Data Analysis

5.2.1.1 *Genetic analysis of microsatellite data*

To examine how genetic diversity was partitioned within and between populations Nei's (1987) gene diversity statistics were calculated in FSTAT v.2.9.3.2 (Goudet 2001). This provides a measure of total genetic diversity over all populations (H_T), the proportion of genetic diversity within populations (H_S) and a measure of genetic differentiation between populations (G_{ST}). G_{ST} is Nei's (1973) estimator of the fixation index F_{ST} and is a frequently used measure of genetic differentiation (Balloux & Lugon-Moulin 2002; Lowe *et al.* 2004). FSTAT was also used to test population differentiation over each locus and overall using the log-likelihood statistic *G*. Hardy-Weinberg equilibrium was not assumed, so the test was based on randomising genotypes among samples rather than alleles; 1000 randomisations were carried out. The log-likelihood statistic *G* is a powerful method for testing whether levels of differentiation are significant (Balloux & Lugon-Moulin 2002).

To estimate genetic divergence between populations Nei's (1972) genetic distance was calculated. This is the most widely used approach for co-dominant data and is based on the probability that two alleles, chosen at random from two different populations, will be identical, relative to the proportion of two alleles chosen from the same population being identical (Lowe *et al.* 2004). Nei's (1972) genetic distance was calculated for all population pairs using the PHYLIP v3.6a package (Felsenstein 1989). Programs within the PHYLIP package were then used to create unrooted consensus trees showing the relationships between populations. Unrooted trees were generated using the unweighted pair group method of clustering (UPGMA). To test the robustness of tree topologies,

Seqboot was used to generate 1000 bootstrap replicates of the allele frequency data that were then analysed in *Gendist*. *Neighbour* was used to create tree topologies for all replicates and *Consense* to produce a consensus tree using a majority rule and majority rule extended approach. Majority rule only supports nodes that have bootstrap values above 50%, whilst the extended approach shows bootstrap support for all of the nodes. The resulting trees were plotted using Treeview v.1.6.6 (Page 1996).

Isolation by distance was investigated by calculating the geographic distance between each population pair using a program written by C. Ford (Spirent Communications plc, source code available on request). Geographic distance was compared to Nei's (1972) genetic distance using IBDWS v. 3.04 (Jensen *et al.* 2005). This examined relationships between all combinations of untransformed data and log (genetic distance) and log (geographic distance). The significance of relationships was determined using Mantel tests based on 10,000 randomisations of the data. Mantel tests overcome problems of non-independence in repeated comparisons of data points. The slope and intercept of relationships were calculated in IBDWS by Reduced Major Axes regression (RMA). Bohanak (2002) considered RMA regression more appropriate than standard ordinary least squares regression, as it is less biased when the independent variable is measured with error. All genetic measures were calculated for ramets and genets, so that the effect of clonal growth could be seen.

5.2.1.2 *Morphological differences*

Discriminant analysis in SPSS v.14 was used to determine which morphological characters discriminated best between populations and to provide a visual representation of population differences. Discriminant analysis was carried out separately on plants from 2003 and 2004. This technique is particularly sensitive to outliers (Tabachnick & Fidell 2007), so individual discriminant plots were viewed for each population and outlying values removed. For the 2004 data, this resulted in the removal of five plants out of 395,

which were from populations ILB, EAC, EMS, IBL and ILG. For 2003, four out of 664 plants were removed, from populations WDB, WWM, EAC and EBB.

Correlations were examined between each of the morphological characters and the habitat variables used in Chapter 4. This was used to investigate potential links between the offspring's morphology and the parent's environment, thus providing some indication of possible maternal effects (Ouborg *et al.* 1991).

5.2.1.3 *Crossing experiment*

To investigate the effect of inbreeding versus outbreeding on seed production and germination, the following variables were determined from each of the crossed plants. a) The number of hard seeds per capitulum was counted. b) The proportion of hard seeds per capitulum was calculated as the number of hard seeds per capitulum / the total number of shrivelled and hard seeds per capitiulum. c) The percentage of hard seeds that germinated per capitulum was determined.

To examine the differences between the crossing treatments, one-way ANOVAs with post-hoc Tukey tests were performed using SPSS v14. The number of hard seeds per capitulum was square-root-transformed, proportion of hard seeds per capitulum log-transformed and percentage germination arcsine-transformed prior to analysis to improve normality and homogeneity of variance.

In addition, the mean number of hard seeds, proportion of hard seeds and percentage germination was calculated for each population pair that was crossed. The geographic and genetic distance between these population pairs was determined and Pearson's correlation coefficients calculated between seed production and genetic and geographic distance. Percentage germination, genetic and geographic distance were square-root-transformed to improve normality and homogeneity of variance and one-tailed tests carried out as it was expected that increasing genetic and geographic distance would decrease seed production and germination.

5.3 Results

5.3.1 Partitioning of genetic variation within *Cirsium dissectum*

The greatest amount of genetic diversity in *C. dissectum* was found within, rather than between populations (**Table 5.1**). Analysis at the ramet and genet level resulted in very similar values, total diversity (H_T) and diversity within a population (H_S) was slightly lower and differentiation (G_{ST}) slightly higher when ramets were analysed compared to genets. Tests of population differentiation using the log likelihood statistic G, were significant at the < 0.001 level for all loci and overall for ramets and genets (data not shown).

Table 5.1 Nei's (1987) gene diversity statistics calculated using FSTAT v. 2.9.3.2 (Goudet 2001). (H_T is the overall gene diversity and G_{ST} is the coefficient of gene differentiation (an estimator of the fixation index F_{ST}), H_T and G_{ST} were calculated so that they were independent of the number of samples. H_S is the within sample gene diversity).

	Ramets			Genets		
Locus name	H_T	H_S	G_{ST}	H_T	H_S	G_{ST}
Caca 1	0.499	0.411	0.177	0.499	0.413	0.173
Caca 4	0.419	0.299	0.287	0.423	0.304	0.281
Caca 5	0.852	0.661	0.224	0.853	0.668	0.217
Caca 7	0.539	0.431	0.201	0.538	0.432	0.196
Caca 16	0.466	0.295	0.368	0.462	0.299	0.354
Caca 22	0.650	0.435	0.330	0.647	0.440	0.319
Caca 24	0.630	0.383	0.391	0.635	0.387	0.391
Mean	**0.579**	**0.416**	**0.283**	**0.580**	**0.420**	**0.276**
SE	**0.021**	**0.018**	**0.012**	**0.021**	**0.018**	**0.012**

5.3.2 Population differentiation and isolation by distance

Appendix F contains matrices of geographic and Nei's (1972) genetic distance between the populations surveyed. Nei's (1972) genetic distance was used to construct consensus trees that illustrate the relationships between populations (**Figure 5.1** and **Figure 5.2**). The degree of resolution was lower when ramet data was used compared to genets. Comparison of the majority rule and majority rule extended trees showed that the ramet tree was similar to that for the genets but had a lower bootstrap support for most nodes.

The majority rule consensus tree for genets (**Figure 5.1**a) showed structuring of populations along geographical lines. There was considerable bootstrap support (98%) for the division of populations between Ireland and Britain. Within the Irish populations, Lough Bunny (ILB), Lough Gealain (ILG) and Lough Corrib (IAR) clustered together. ILB and ILG are both populations from the Burren whilst IAR is relatively close. Doagh Lough (IDL) and Lough Lattone (ILL) clustered together and these are also relatively close geographically. Within the British populations, the pattern of geographical structuring continued. The Welsh populations were separated from the English with a bootstrap support of 53% and Cefn Cribwr (WCG) and Kenfig (WKF) clustered together genetically and are also very close geographically. The majority rule consensus tree showed little resolution between most of the English populations, although Wicken Fen (EWF) was genetically and geographically distant from the other English populations. The position of Braunton Burrows (EBB) did not fit the geographic pattern; it was separated from the rest of the English populations genetically but is only 24 km away from Mambury Moor (EMM). The majority rule extended tree (**Figure 5.1**b) showed more geographic structuring within the English populations, Knowstone Moor (EKS) and Meshaw Moor (EMS), both rhos pastures in North Devon, clustered together with a bootstrap support of 41%, whilst the two New Forest populations, Rans Wood (ERW) and Marlpitt Oak (EMO), clustered with a bootstrap value of 49%. It should be noted however that nodes with bootstrap support lower than 50% are less reliable.

In order to investigate whether the apparent clustering along geographic lines revealed in the trees related to significant correlations between geographic and genetic distance, the program IBDWS v. 3.04 (Jensen *et al.* 2005) was used. Highly significant positive relationships existed between all combinations of untransformed and transformed data and ramets and genets, although the relationships were consistently, slightly lower when ramets were used compared to genets (**Table 5.2**). The highest correlation was seen

between geographic and log(genetic) distance for the genet data with an r (standardized Mantel test statistic) of 0.723 and an R^2 of 0.530 (**Figure 5.3**).

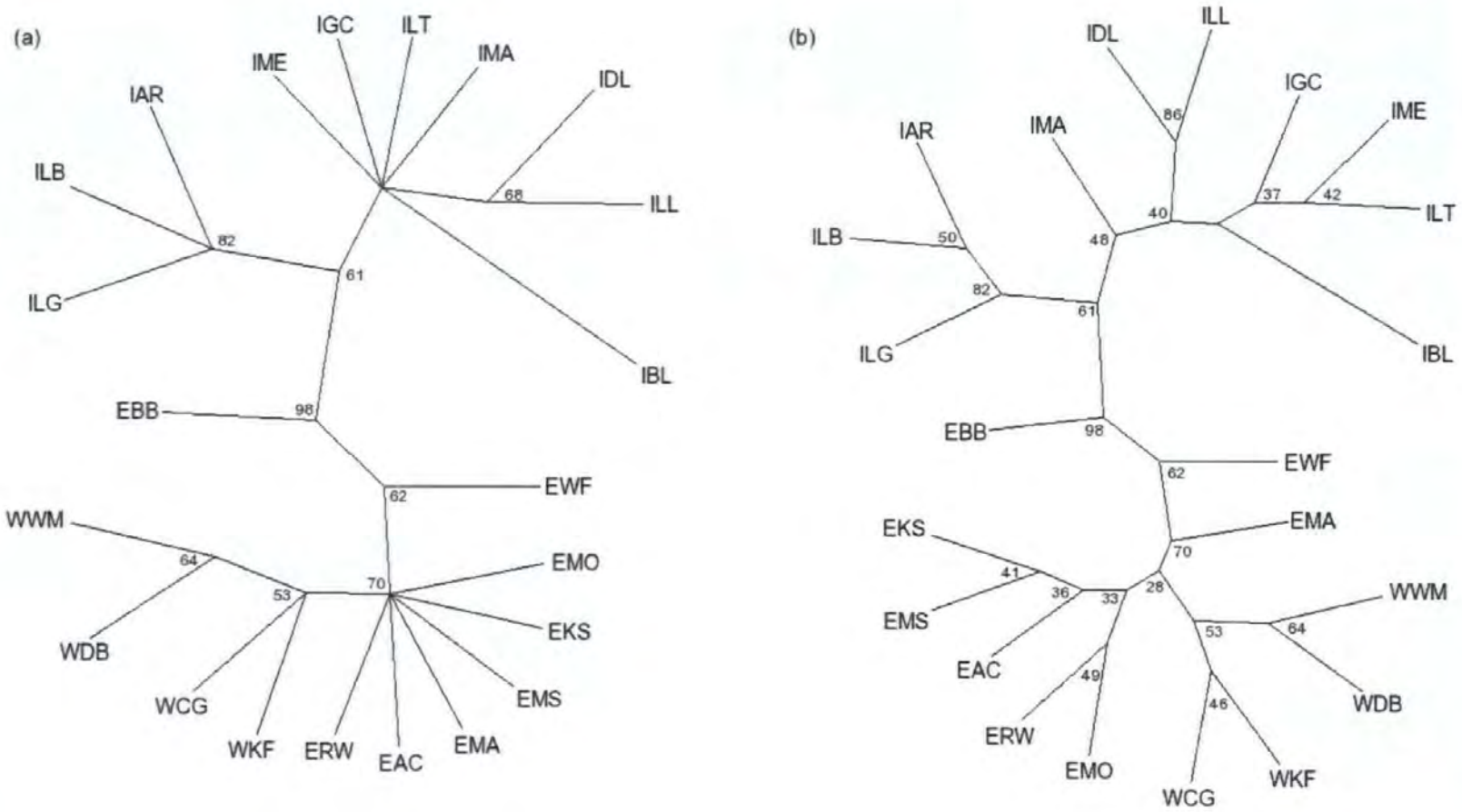


Figure 5.1 Unrooted consensus trees showing the relationships between populations using genet data, constructed using Nei's (1972) genetic distances and UPGMA clustering. (Numbers are the bootstrap values for the adjacent node and represent the percentage of 1000 trees where populations beyond the node grouped together. (a) majority rule, (b) majority rule extended).

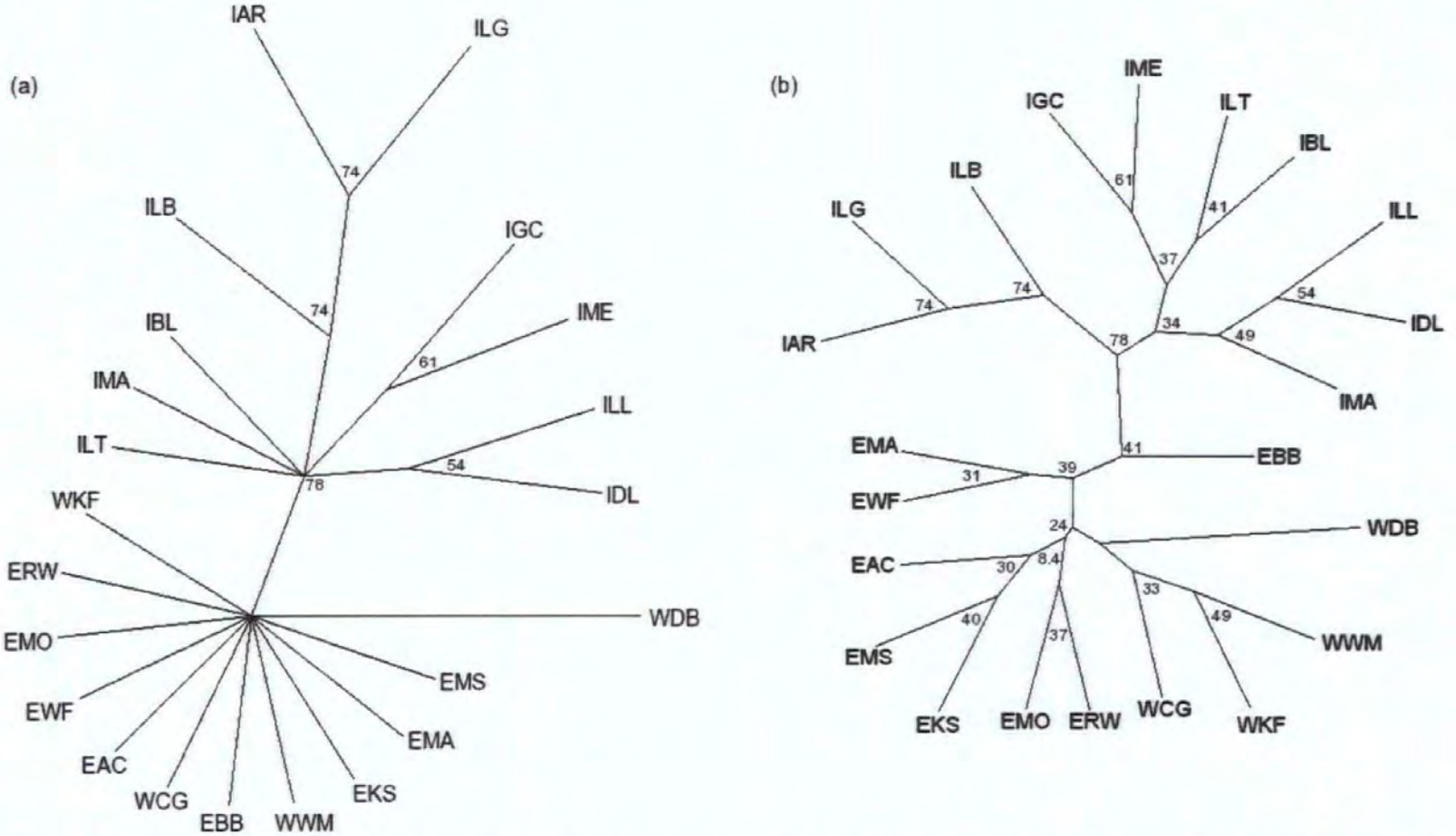


Figure 5.2 Unrooted consensus trees showing the relationships between populations using ramet data, constructed using Nei's (1972) genetic distances and UPGMA clustering. (Numbers are the bootstrap values for the adjacent node and represent the percentage of 1000 trees where populations beyond the node grouped together. (a) majority rule, (b) majority rule extended).

Table 5.2 Isolation by distance using IBDWS v. 3.04 (Jensen *et al.* 2005). (Relationships between all combinations of untransformed data and log (genetic distance) and log (geographic distance). Significance of relationships was assessed using a Mantel test, *Z* is the test statistic, *r* provides a standardized *Z* that ranges from –1 to 1. *** represents a one-tailed *P*-value of < 0.001).

	Ramets			Genets		
N = 231	***Z***	***r***	**R^2**	***Z***	***r***	**R^2**
Genetic vs. Geographic	33238.8	0.571***	0.326	32753.4	0.583***	0.339
Genetic vs. Log(geographic)	211.9	0.526***	0.277	208.9	0.541***	0.292
Log(genetic) vs. Geographic	-29531.7	0.722***	0.522	-29811.2	0.723***	0.530
Log(genetic) vs. Log(geographic)	-284.0	0.693***	0.480	-285.6	0.703***	0.494

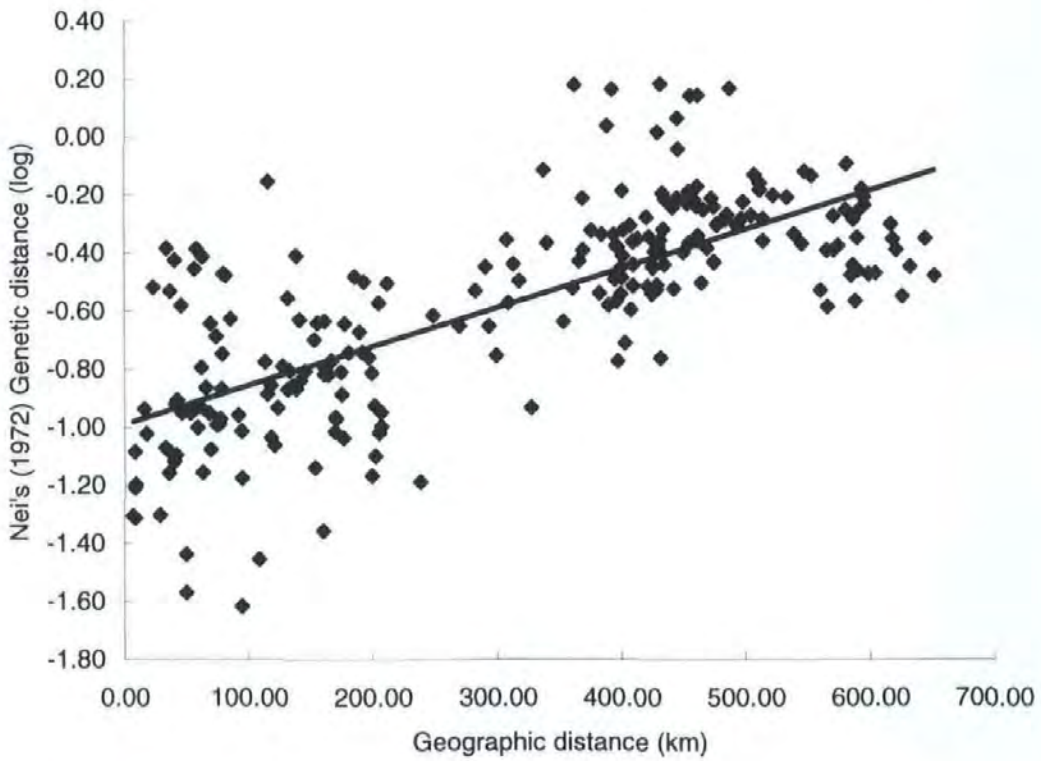


Figure 5.3 Nei's (1972) genetic distance (log) as a function of geographic distance for all combinations of 22 populations. (Data shown are for genets. $r = 0.723$, $R^2 = 0.53$, $P = < 0.001$).

5.3.3 Comparison between genetic distances using microsatellite markers and morphological variation.

Correlations between habitat variables and morphological characters were carried out to test for possible maternal effects. For the nine populations surveyed in 2003, the number of ramets was significantly correlated with the amount of bare soil in the parent site, whilst for the 14 populations sampled in 2004, ramet number was correlated with vegetation height. None of the other characters showed any correlation with the habitat variables. Ramet number was therefore removed from the discriminant analysis.

The discriminant analysis for the 2004 plants from 14 populations showed that the British populations formed a tight cluster that was clearly separated from the Irish populations (**Figure 5.5**). The Irish populations showed considerable differentiation from each other. The two populations from the Burren, Lough Bunny (ILB) and Lough Gealain (ILG) were separated from each other and the remaining Irish populations along function 2. This function was most strongly influenced by the petiole:leaf length ratio with additional contributions from the petiole length, the angle of the leaf at the base and apex, the leaf width:leaf length ratio and the leaf width (**Table 5.3**). The Burren populations and ILG in particular therefore had leaves with longer petioles relative to the leaf length and slight differences in leaf shape compared to all the other populations.

All of the Irish populations were differentiated along function 1. This correlated most strongly with how pinnatifid the leaves were, with additional contributions from the number of main veins and the degree of undulation of the leaves (**Table 5.3**). The Irish populations showed some geographical structuring along function 1 as ILG and ILB were next to each other but Bleach Lough (IBL) and Giant's Causeway (IGC) were also next to each other, even though they are the two most distantly separated populations geographically. Meencargagh (IME) was the most extreme population along function 1, and had considerably more pinnatifid leaves compared to the other populations. The

difference in IME leaves was clearly identifiable within the plants growing within the net-tunnel (**Figure 5.4**).

The 2003 plants from nine British populations showed little differentiation (**Figure 5.6**), Drostre Bank (WDB) and Braunton Burrows (EBB) showed the greatest degree of separation along function 1. This function was correlated to the angle at the base and apex of the leaf, petiole:leaf length ratio, petiole length, leaf width and leaf width:leaf length ratio; it therefore appears to correspond to function 2 from the 2004 plants (**Table 5.3**). Function 2 (for 2003) was most strongly related to the leaf width:leaf length ratio and the apex angle of the leaves but the correlations were lower.

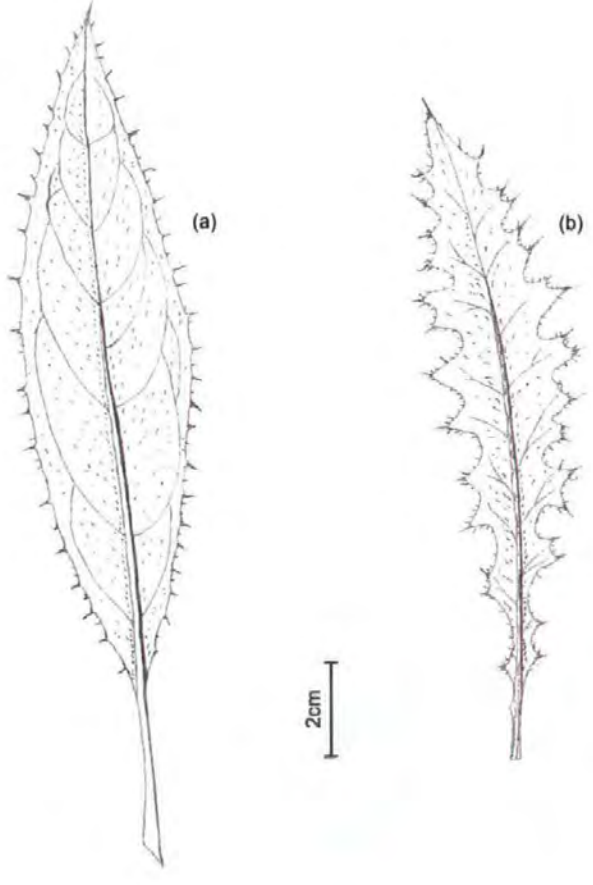


Figure 5.4 Morphology of leaves grown in standard conditions. (a) leaf from Mambury Moor (EMM) this represents the 'typical' leaf form. (b) leaf from Meencargagh (IME) this shows irregular leaf dissection.

Table 5.3 Pooled within-group correlations between discriminating variables and standardized canonical discriminant functions for plants grown in 2004 and 2003.

	2004 plants (England, Wales and Ireland)		2003 plants (England and Wales)	
Character	**Function 1**	**Function 2**	**Function 1**	**Function 2**
Pinnatifid ratio	0.811	-0.048	-0.236	-0.247
Number of veins	-0.550	-0.118	-0.126	-0.378
Undulation (mm)	-0.542	0.365	-0.029	0.327
Petiole: leaf length ratio	0.302	-0.800	0.455	0.225
Petiole length (mm)	0.213	-0.554	0.450	0.187
Angle at base of leaf (degrees)	0.166	0.462	-0.732	0.205
Angle at apex of leaf (degrees)	0.267	0.454	-0.468	0.463
Leaf width widest: leaf length ratio	0.082	0.461	-0.639	0.461
Spine length (mm)	-0.165	-0.025	0.168	0.139
Leaf length x width (mm^2)	-0.093	0.243	-0.196	0.136
Leaf length (mm)	-0.100	0.052	0.160	-0.070
Number of leaves	-0.134	0.212	-0.185	0.395
Leaf width (mm)	-0.058	0.445	-0.480	0.304

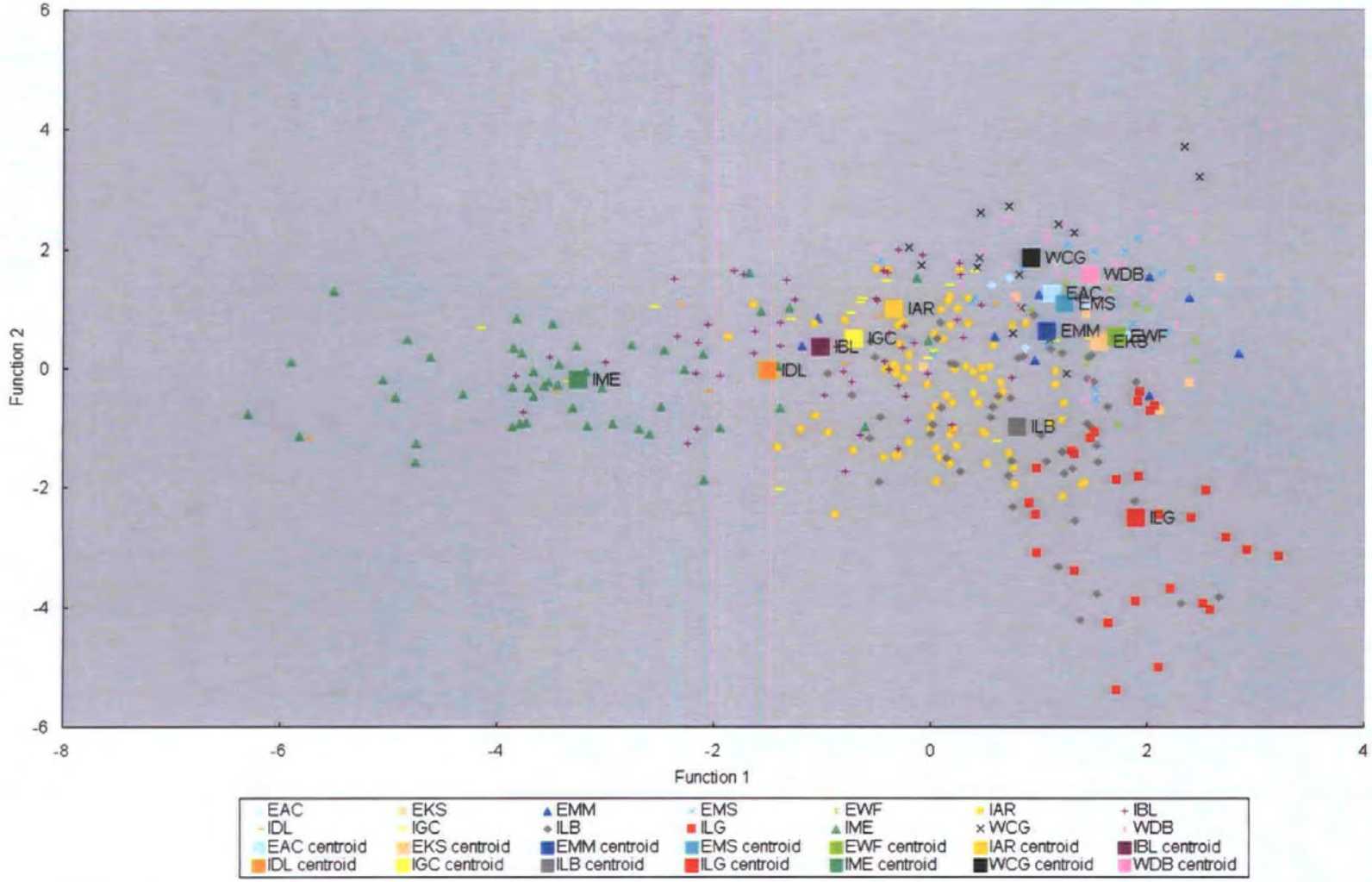


Figure 5.5 Discriminant plot showing relationships between plants grown from seeds collected in 2004 from 14 populations. (Thirteen morphological characters were measured on each plant. Individual datapoints represent individual plants and large squares represent population centroids).

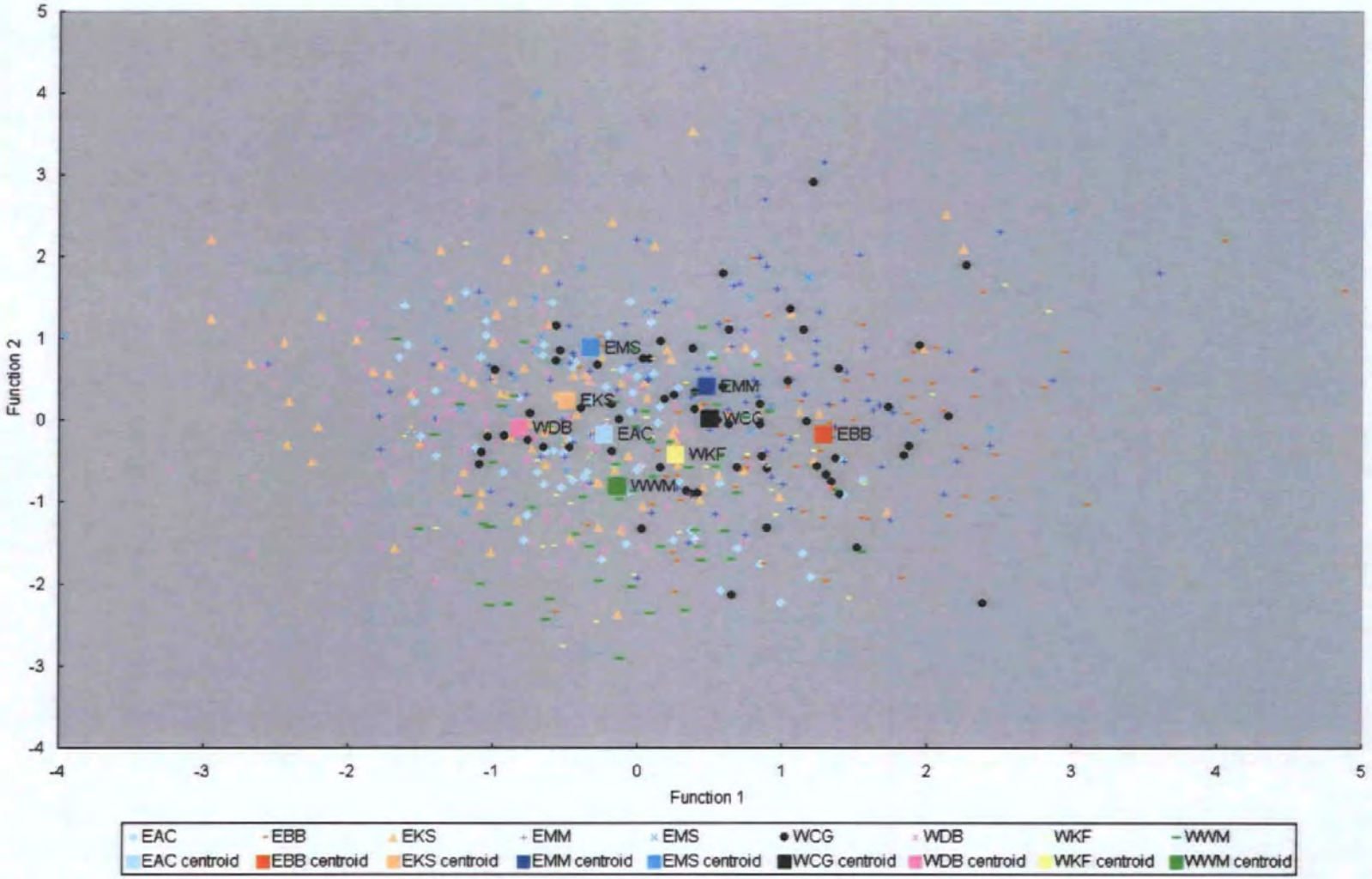
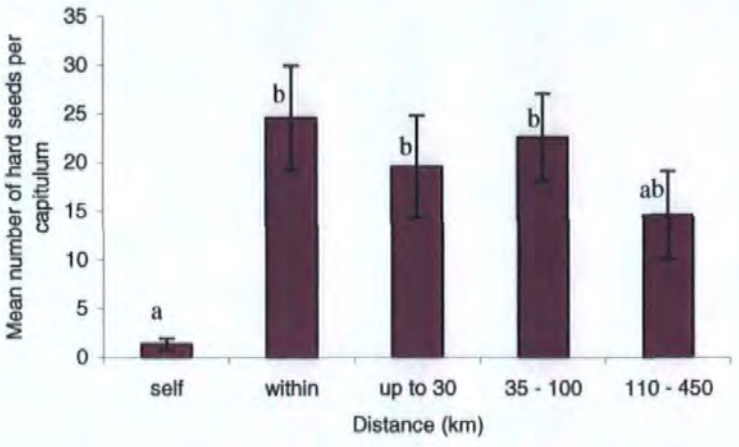


Figure 5.6 Discriminant plot showing relationships between plants grown from seeds collected in 2003 from 9 populations. (Thirteen morphological characters were measured on each plant. Individual datapoints represent individual plants and large squares represent population centroids).

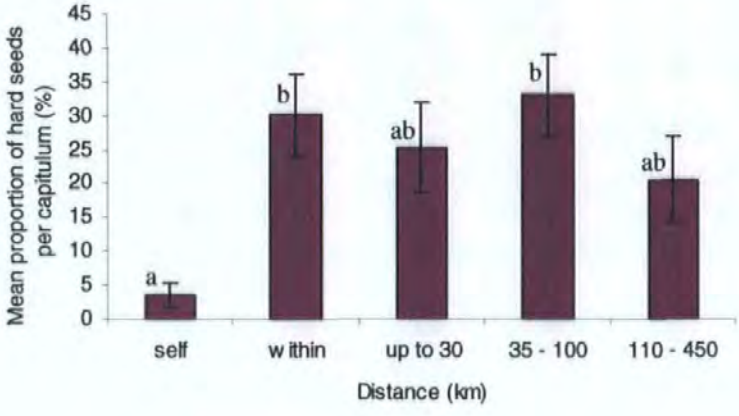
5.3.4 The effect of inbreeding and outbreeding on seed production and germination

Capitula that were selfed produced significantly less hard seeds compared to capitula that were crossed, and the number of hard seeds decreased relative to the number of shrivelled, undeveloped seeds (**Figure 5.7**). No germination of selfed seeds was seen. There were no significant differences between the number, proportion and germination of seeds when plants were crossed from within the same population or from a population up to 450 km away. There was a trend however, that the number and proportion of hard seeds per capitulum was lower at crossing distances of 110 to 450 km.

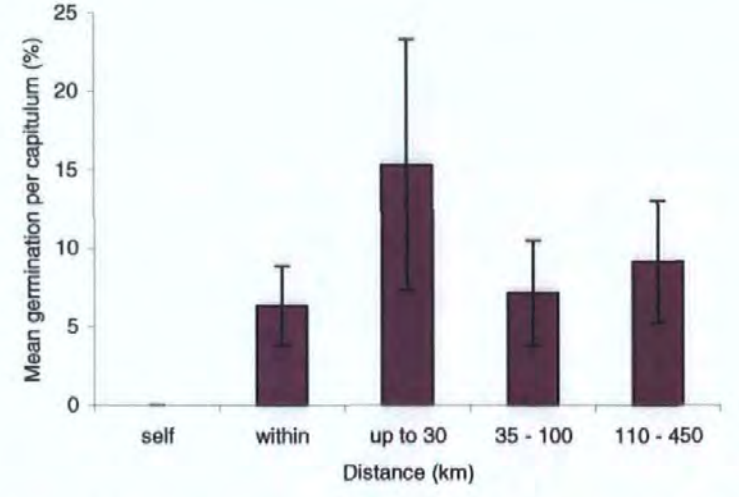
There were no significant relationships between geographic distance and seed production or germination (**Figure 5.8**). Similarly, there were no significant differences between seed production or germination and genetic distance but there were trends suggesting that the number and proportion of hard seeds per capitulum decreased at greater genetic distances.



Mean square (between groups)	37.228
df (between groups)	4
Mean square (within groups)	8.119
df (within groups)	114
F-ratio	4.585
P-value	0.002



Mean square (between groups)	2.394
df (between groups)	4
Mean square (within groups)	0.536
df (within groups)	114
F-ratio	4.465
P-value	0.002



Mean square (between groups)	0.054
df (between groups)	4
Mean square (within groups)	0.047
df (within groups)	72
F-ratio	1.159
P-value	0.336

Figure 5.7 Seed production (measured as the mean number of hard seeds and the mean proportion of hard seeds relative to shrivelled seeds per capitulum) and germination (measured as the percentage of hard seeds that germinated per capitulum) for plants grown in standard common garden conditions and either selfed, crossed within a population or crossed with different populations at a range of geographic distances. All of the crosses were performed by the author; A. Whotton (University of Plymouth) counted and germinated the seeds. The error bars are standard errors. Results of a one-way ANOVA and *post-hoc* Tukey test are shown, calculated using SPSS v14. Treatments that do not share a letter are significantly different.

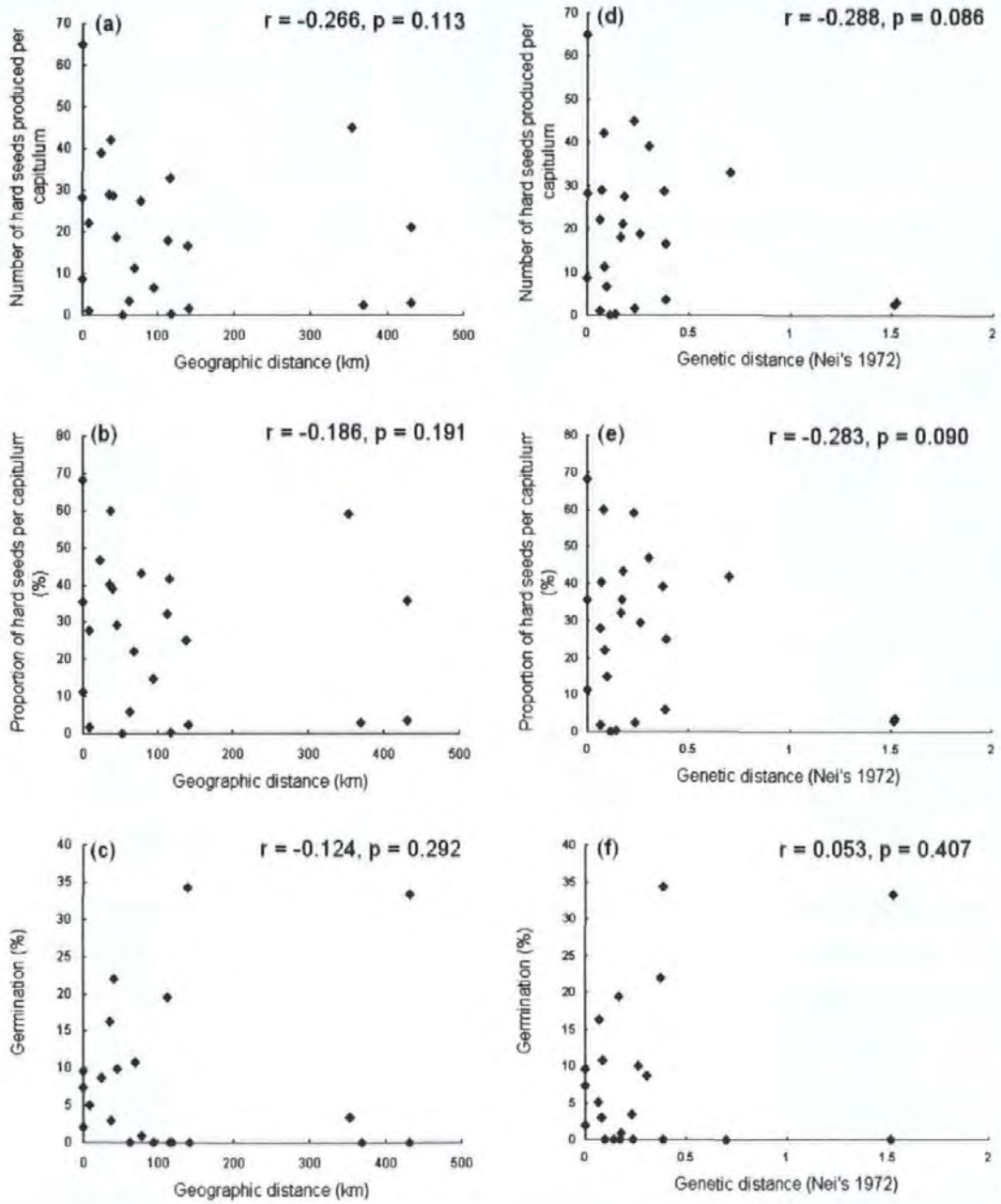


Figure 5.8 Relationships between seed production, germination, geographic and genetic distance (Nei's 1972) for plants grown in standard conditions and subjected to experimental crosses within populations and at various geographic distances. (r = Pearson's correlation coefficient).

5.4 Discussion

5.4.1 Genetic variation in *Cirsium dissectum*

Within *C. dissectum*, 58% of genetic variation was partitioned within populations and 42% among populations. Although diversity was higher within than between populations, many of the G_{ST} values were greater than 0.25, which is considered to represent very great differentiation (Hartl & Clark 2007). Smulders *et al.* (2000), using AFLP markers, also found more genetic variation within, compared to between, populations, although a lower level of differentiation was recorded (Φst 0.108). Smulders *et al.* (2000) only sampled five populations with a maximum distance of approximately 250 km, whilst this study sampled 22 populations with a maximum distance of 650 km, so this may help to explain the differences observed. Kay and John (1994) using allozymes reported that they were unable to calculate a G_{ST} value for *C. dissectum* due to very low levels of polymorphism.

Hamrick & Godt (1990) related levels of differentiation to life history characteristics. They recorded an average G_{ST} of 0.510 for selfing species, 0.216 for animal pollinated species with a mixed mating system and 0.197 for animal pollinated, outcrossing species. Care should be taken when using meta-analyses such as these, due to differences in molecular markers used, sampling strategy and methods for calculating statistics; nevertheless these studies are valuable in providing context for gene diversity statistics (Lowe *et al.* 2004). Comparing G_{ST} values for *C. dissectum* suggests a mixed mating system for this species. This result corresponds to the positive inbreeding co-efficients described in Chapter 4 that were also indicative of a species that combined outcrossing with some selfing.

5.4.2 Population differentiation in *Cirsium dissectum*

The unrooted UPGMA trees produced using neutral molecular markers (microsatellites) and the discriminant analysis using morphological characters provided broadly similar

results. Both showed some structuring of populations according to their geographical relationships and this was confirmed by a significant relationship between genetic and geographic distance. Kay & John (1994) also found some evidence of geographical structuring in *C. dissectum* using allozymes.

Isolation by distance is often found in plant populations and can be related to the re-colonisation of the British Isles after the last glaciation (Jump 2002; Bockelmann *et al.* 2003). As plants colonised further from their refugial populations, gene flow gradually decreased and genetic isolation increased (Chauvet *et al.* 2004). The high level of differentiation now seen in *C. dissectum* is likely to have been reinforced by more contemporary factors such as habitat destruction, leading to increased isolation of populations (Schaal & Leverich 1996).

An exception to the geographical structuring found using the microsatellite markers was Braunton Burrows (EBB) which showed considerable genetic differentiation compared to the other British populations. One possible explanation for this is that EBB is a relatively small population, and loss of genetic diversity and genetic drift will lead to increased differentiation in small, isolated populations (Barrett and Kohn 1991, Frankham *et al.* 2002). Levels of genotypic variation (G/N) and allelic richness were however lower in plants from EBB than expected for a population of its size (Chapter 4 & Chapter 5) and this means that current population size alone cannot explain the high level of differentiation. Another explanation is that the EBB population was established with a very small number of plants and that founder effects have led to greater differentiation (Solé *et al.* 2004). This suggests that no gene flow occurs between EBB and other local populations, as this would reduce differentiation. This seems unlikely, as EBB is only 24 km away from Mambury Moor (EMM). A further explanation for the low level of genetic and genotypic diversity and high differentiation are strong selection pressures working within this population (Solé *et al.* 2004). Microsatellite markers are considered to be

selectively neutral but if the plants at EBB are locally adapted, then even if gene flow does occur from a nearby population in a different habitat the foreign plants may be unable to survive, leading to increased genetic differentiation. EBB is a dune-slack site, whilst all the other English populations are sampled from rhos pastures and heaths. This is a very different habitat type and local adaptation may therefore be expected. Bockelmann *et al.* (2003) showed genetic differentiation by habitat for the salt marsh grass, *Elymus athericus*, and thus differentiation by habitat is feasible in *C. dissectum*. In contrast, however, the *C. dissectum* plants from the other dune slack site, Kenfig (WKF), were most genetically similar to the rhos pasture closest to them, Cefn Cribwr (WCG), so differentiation between dune slacks and rhos pastures was not apparent in this case. Within the discriminant analysis plots, the plants from EBB did show some differentiation but still overlapped with the other populations. Although not conclusive, local adaptation is the most likely explanation for the genetic differences in EBB plants and this hypothesis could be tested using reciprocal transplant experiments.

5.4.3 Differentiation in Ireland compared to Britain.

Nei's (1972) genetic distances showed similar levels of differentiation in populations located in Ireland and Britain. The mean Nei's (1972) genetic distance between all of the Irish populations was 0.119 (genets), whilst the mean genetic distance between the British populations was 0.216 (genets). The discriminant analysis plot, however, showed greater variation between the Irish populations, compared to the British populations.

Populations from the Burren, Lough Gealain (ILG) and Lough Bunny (ILB) had longer petioles compared to the other populations and these differences may reflect geographic distance, as these two populations are closest to each other. It may also reflect local adaptation, as these two populations were both growing on limestone and the ILG plants in particular had a very different environment relative to the other populations,

growing between the limestone pavement right up to the shores of Lough Gealain (see photo in site description, Appendix A).

The character that most strongly differentiated all of the Irish populations was the degree of leaf dissection (pinnatifid ratio). Populations in Ireland may therefore have genetic differences that affect this morphological trait. Chapter 1 described the forms of *C. dissectum* recognised by Rouy (1905) and these differed in how pinnatifid the leaves were, but Sell & Murrell (2006) did not recognise any different forms within the British Isles. The variation in leaf dissection did not appear to relate to geographical differences.

Meencargagh (IME) showed the most pinnatifid leaves under standard conditions and these were clearly identifiable from the plants from other populations growing within the net-tunnel. In the IME site, however, the leaves were not pinnatifid. It is possible that the leaf shape was due to introgression with *C. palustre*, although no *C.* x *forsteri* were found flowering at this site and this would suggest very high levels of hybridization. IME was not particularly differentiated from the other Irish populations based on Nei's (1972) genetic distance. Further investigation would be required in order to explain this result more fully and the effects of local adaptation and hybridization are two interesting areas for further work.

Maternal effects could be responsible for some of the morphological differences observed but these are most likely to be expressed early in the life-cycle in the seedling and juvenile stages (Ouburg *et al.* 1991; Picó *et al.* 2004). No seed characters were used in this study and plants were one-year-old when they were measured. This along with the tests for relationships between the morphological characters and habitat variables should reduce the possibility of maternal effects.

5.4.4 Inbreeding versus outbreeding

The proportion of hard seeds produced in selfed plants was only 10.6% that of plants crossed within a population and none of the selfed seeds germinated. This reduced seed production may be caused by self-incompatibility or early acting inbreeding depression.

A single-locus sporophytic self-incompatibility mechanism is characteristic of members of the Asteraceae (De Nettancourt 1977). Plants that share the same S-alleles are unable to produce seeds, so populations require a large number of S-alleles to be segregating, so that there are many compatible mating types. In small populations, S-alleles can be lost through genetic drift and the subsequent lack of mating types can make the species vulnerable to extinction (Demauro 1994). The loss of S-alleles in small populations and subsequent decline due to lack of mating types has been recorded in *Hymenoxys acaulis* (Demauro 1994) and *Rutidosis leptorrhynchoides* (Young *et al.* 2000). Luijten *et al.* (2002) found that selfing produced 27% of the seed production, compared to interpopulation crosses in *Arnica montana* and a similar result was observed after selfing in *Hypochaeris radicata* (Picó *et al.* 2004). In both cases, the reduction in seed production was attributed to a sporophytic self-incompatibility mechanism and all of these species belong to the Asteraceae.

A sporophytic self-incompatibility system has not been reported for *Cirsium* species however and typically selfing produces some viable seeds (Piggot 1968 *C. acaule*; Klinkhamer & de Jong 1993 *C. vulgare*; Ohashi & Yahara 1998 *C. purpuratum*; McEachern *et al.* 1994 *C. pitcheri*). Methods to reduce selfing include being incompletely dioecious in *C. arvense* (Solé *et al.* 2004), gynodioecious in *C. acaule* (Pigott 1968) and protandrous in *C. purpuratum* (Ohashi & Yahara 1998), whilst *C. pitcheri* (Weller 1994) has a mixed mating system with outcrossing rates ranging from 35 to 88%. A sporophytic self-incompatibility system is therefore unlikely to be found in *C. dissectum*; the

inbreeding coefficients described in Chapter 4 and the G_{ST} value all suggest a mixed mating system.

If low seed production is not caused by a self-incompatiblity mechanism, then it is likely to be a product of inbreeding depression. The number of hard seeds produced, compared to shrivelled seeds, provides an estimate of the seed/ovule ratio and this can be a sensitive measure of inbreeding depression (Demauro 1994). In outcrossing species, inbreeding depression is often expressed either early in the life-cycle, during seed development, or late, during the plants growth or reproduction (Luijten *et al.* 2002). This suggests that inbreeding depression could be occurring in *C. dissectum.*

There is no strong evidence for outbreeding depression in *C. dissectum* but there are possible trends. Geographic distance did not show any significant relationship with seed production, although less seeds were produced at crossing distances of 110 to 450 km. There are marginally significant negative correlations between the total number and proportion of hard seeds and genetic distance. Within the crossing treatments there were substantial variations in seed production depending on the populations crossed. Bailey & McCauley (2006) conducted a similar experiment on *Silene vulgaris* and found comparable results in that selfing caused a large reduction in seed production, whilst crosses between geographical regions showed some reduction. They examined the results for different geographical regions separately and showed substantial variation between regions. It seems therefore that a simple relationship between genetic distance and seed production is unlikely. Indeed, it is to be expected that levels of genetic diversity within a population and the amount of inbreeding will influence the effects of inter-population crosses. In addition, if plants from different habitats are crossed this may influence seed production if local adaptation is present and finally, maternal effects cannot be discounted. Sample sizes were not large enough in this experiment to use either population size, within population genetic diversity or habitat as a treatment.

Notwithstanding the observations above, some evidence for outbreeding depression in the F1 generation of *C. dissectum* was observed. Outbreeding depression in the F1 generation has been seen in some other species (Waser & Price 1994; Fischer & Matthies 1997; Montalvo & Ellstrand 2001; Bailey & McCauley 2006), whilst in other cases heterosis is seen (Luijten *et al.* 2002; Vergeer *et al.* 2004). Outbreeding depression is potentially less likely to be expressed in the F1 generation, as all the individuals are heterozygous at a high number of loci and more likely to be expressed in later generations when deleterious interactions between homozygous loci become exposed (Luijten *et al.* 2002, Edmands 2007). A limitation of the present study is that plants were only observed for a small part of the F1 generation, whilst ideally plants would be observed over a number of generations. Fenster & Galloway (2000a,b) observed heterosis in the F1 and outbreeding depression in the F2 and F3 in the outcrossing annual *Chamaecrista fasciculata.*

Effects of inbreeding and outbreeding may also depend on the environment within which plants are found. The expression of inbreeding depression can be environment dependent and may be magnified under stressful conditions. It is also preferable to grow plants in a population context, so that the effects of other plants can be observed (Bailey & McCauley 2006). Fenster & Dudash (1994) recommend investigating the effects of inbreeding and outbreeding by comparing the performance of plants over a number of generations in each parental source population and in novel environments. Time constraints prevented a more detailed investigation of inbreeding and outbreeding in this study. The results suggest, however, that inbreeding depression and possibly outbreeding depression both occur in *C. dissectum.*

5.4.5 Conservation implications

The high level of differentiation in *C. dissectum* means that more populations would need to be conserved in order to capture the same amount of genetic variation relative to a less

differentiated species (Hamrick *et al.* 1991). As *C. dissectum* shows strong isolation by distance, conserving populations throughout its geographic range would be an effective method. Populations such as Braunton Burrows (EBB), that may show local adaptation, should also be priorities for conservation.

When considering the source of seed to use in restoration programmes, outbreeding depression was possibly observed and this showed a greater relationship with genetic rather than geographic distance. Given the high levels of genetic differentiation and the possibility of outbreeding depression, a more conservative approach that does not mix distant populations should be considered. It is suggested that in restoration projects for *C. dissectum* the closest source of seed should be used. However, the negative effects of inbreeding were pronounced, so it is not advisable to use small, local populations. Instead, collecting plant material from a number of local populations would help to maximise genetic diversity. The possible observation of local adaptation in population EBB and some of the Irish populations suggests that seed should come from a similar habitat. The source of plant material to use in restoration projects is discussed further in Chapter 6.

Chapter 6

6 General discussion and further work.

The overall aim of this study was to examine the ecology and genetics of *C. dissectum* within the British Isles and to consider the implications of the results for its conservation. This was achieved by investigating site characteristics, plant communities and reproductive biology and integrating these findings with the published literature on the species. Levels of genetic diversity within and between populations were examined. Within populations, relationships between genetic diversity, population size, fitness and habitat quality were considered; between populations, levels of genetic differentiation were explored using microsatellite markers and morphological traits. The effects of inbreeding and outbreeding were investigated using crossing experiments. This discussion summarises these findings and considers the implications of this research for the conservation of *C. dissectum*. Possible areas of further research are then described.

6.1 The ecology and genetics of *C. dissectum*

6.1.1 Site characteristics

Within this study, *C. dissectum* was found in rhos pastures, fens, wet heaths and dune slacks, characteristically on peaty soils, although the dune slacks and some heaths were predominantly sandy (Chapter 3). Soil mineral nutrient status was characterised by low phosphorus and non-limiting concentrations of potassium. Calcium was often abundant, whilst total nitrogen and organic matter varied from the low concentrations found in sand dunes to high levels in the peaty sites. These findings are consistent with other studies that have examined soil nutrients in *C. dissectum* sites (Ross 1999; Goodwin 1995; Wheeler & Shaw 1987). All of the *C. dissectum* sites measured were acidic but never below 4.5, probably due to aluminium and ammonium toxicity at pH levels below 4.5 (de Graaf *et al.* 1997; de Graaf *et al.* 1998; Lucassen *et al.* 2002; Dorland *et al.* 2003). It was not possible to sample for available nitrogen in this study but it is assumed that nitrate levels at the sites would be low with nitrification being restricted by the low pH.

6.1.2 Reproductive biology

This study provides the first general description of the reproductive biology of *C. dissectum.* This species has a hemi-cryptophyte basal rosette growth form and reproduces sexually and asexually. A single flowering stem is produced in the second year at the earliest with generally a single capitulum, although sometimes two or three are produced. Each capitulum contains 20 to 160 hermaphrodite florets that are pollinated by a range of bumblebees, butterflies and long-tongued dipteran flies (Kay & John 1994; Chapter 3). Flowers are self-compatible but selfed capitula produce fewer seeds (Chapter 5). Inbreeding coefficients and measures of G_{ST} suggest that *C. dissectum* has a mixed mating system combining outcrossing with some selfing (Chapter 4 and Chapter 5).

Predation of the developing seeds by Tephritid flies is often high and generally each capitulum produces 7 to 83 seeds that vary in mass from 1.3 to 3.6 mg (Chapter 3). Seeds are attached to a pappus and dispersal occurs in dry conditions, with most seeds landing close to the parent plant. Previous studies have shown that germination occurs over a wide range of temperatures and conditions, with highest germination occurring in stratified seeds at 31 °C (Ross, unpublished data). Germination levels were considerably lower in this study, possibly due to the effects of an unknown pathogen (Chapter 3).

For asexual reproduction, *C. dissectum* produces a caudex from which rhizomes are produced that vary in length from 2 to 40 cm. New rosettes are generally produced at the end of rhizomes but can also arise directly from the caudex (Chapter 3; Jongejans 2004). Population dynamics of plants in the Netherlands suggested that clonal growth was the dominant form of reproduction (Jongejans *et al.* submitted) but the genetic analysis of populations from the British Isles described in Chapter 3 showed that, at a 10 x 10 m spatial scale, genotypic diversity was high and most plants had arisen from sexual reproduction. *C. dissectum* often grows in large patches and the analysis of clonal structure showed that these are likely to be composed of more than one clone. Individual clones extended to a length of up to 100 m and may be in the region of 500 years old or more (Chapter 3).

6.1.3 Genetic diversity and the response of *C. dissectum* to different habitat conditions

Very few studies have examined the relationships between genetic diversity, population size, habitat quality and fitness and the author knows of no others where this has been examined in a clonal plant species. Oostermeijer *et al.* (1998), Vergeer *et al.* (2003) and this study have all revealed complex interactions between population size, genetic diversity fitness and habitat quality in plant species and this highlights the importance of considering

genetics and habitat quality together, when investigating the effects of habitat destruction and modification on plants.

Chapter 4 showed that *C. dissectum* responded differently in dissimilar habitats; two habitat extremes can be visualised that affect levels of reproduction, genetic diversity and fitness. At one extreme, sites with tall vegetation and no bare soil had abundant flowering rosettes of *C. dissectum* (Chapter 4). This corresponds with findings in the Netherlands that flowering was greater in more productive sites and in experimental plots containing *C. dissectum* plants that were fertilised (Jongejans *et al.* submitted). The greater level of flowering will not necessarily result in greater successful sexual reproduction however, as bare soil is needed for seedling establishment. In sites with no bare soil, allelic richness was reduced, probably due to the lack of sexual recruitment and this subsequently had a negative effect on plant fitness (Chapter 4). Eventually, in high productivity sites, *C. dissectum* will be out-competed by species such as *Molinia caerulea*, that are able to build up biomass more quickly (Jongejans *et al.* 2006a). The loss of individuals through competition, along with the suppression of sexual reproduction, will reduce population size and levels of genetic diversity, and this will subsequently increase extinction risk through demographic and genetic stochasticity.

At the other extreme, in sites with very short vegetation and abundant bare soil, flowering was suppressed, and this may also prevent sexual reproduction. These sites often had lower levels of phosphorus, and phosphorus is important as higher levels related to greater allelic richness, reduced inbreeding and more germination under standard conditions. Phosphorus may relate to genetic diversity by promoting flowering or by increasing seedling survival (Chapter 4).

Sites with abundant flowering and no bare soil may act as sources for sites where flowering is suppressed but bare soil is available. This may occur in restoration sites, where topsoil is removed next to existing populations but is unlikely to occur in the

wider landscape, as Soons *et al.* (2005) has shown that *C. dissectum* populations in the Netherlands are reproductively isolated from each other.

6.1.4 Genetic differentiation

Levels of genetic differentiation (G_{ST}) were high in *C. dissectum* and this supports the results that suggest relatively low dispersal potential in *C. dissectum* (Chapter 5). Populations showed isolation by distance and were largely structured by their geographic position (Chapter 5). Braunton Burrows (EBB) was an exception to this; it had a greater genetic distance and lower genetic and genotypic diversity than expected for a population of its size and geographic position (Chapter 4 and Chapter 5). This may indicate that this population arose quite recently from a small number of founder members or that strong selection pressures act within this dune slack site. Patterns of differentiation using molecular markers and morphological traits were broadly similar but populations in Ireland showed greater differentiation in morphological traits relative to Britain.

6.2 Conservation

Cirsium dissectum has declined throughout its range and is endangered in Germany and the Netherlands. The British Isles, and Ireland in particular, represent a stronghold for this species and therefore have an international responsibility to protect it (Chapter 1). Although rather specialized in its habitat requirements, it can be abundant in suitable conditions and is not inherently rare. Its decline is principally due to agricultural intensification that has caused loss of sites through drainage and fertilization and a lack of management that has led to succession (Buck-Sorlin 1993; Buck-Sorlin & Weeda 2000;

Preston *et al.* 2002). As an Oceanic Temperate species that depends on a warm, moist climate, global warming may have a particularly adverse effect.

C. dissectum is a key species of Cirsio-Molinietum grasslands, classified in Britain as M24 in the National Vegetation Classification (Rodwell 1991). It has more specialized habitat requirements than the other key species in this community and therefore acts as an important indicator species for the community as a whole (Chapter 1). Beyond this, it is a representative of semi-natural, nutrient-poor and species-rich grasslands that have suffered massive losses throughout Europe. Understanding the ecology of *C. dissectum* can therefore provide information that will help to conserve this species and the community it is found within. It may also provide information that is valuable for the conservation of semi-natural grassland plants. Practical conservation suggestions must be based on a thorough understanding of species ecology and genetics.

6.2.1 Which populations should be conservation priorities?

Cirsium dissectum showed strong geographic structuring and high levels of genetic differentiation, so in order to maximise the conservation of genetic diversity, populations should be conserved throughout the geographic range of the species in the British Isles (Chapter 5). The protection of sites in Ireland is particularly important as *C. dissectum* is still relatively abundant there, especially in western Ireland, and this represents a stronghold for the species. This study has shown that levels of genetic diversity were high in Ireland and plants showed a considerable amount of morphological differentiation when grown in standard conditions (Chapter 5).

Conservation of *C. dissectum* in dune slack communities is also important, as this represents an atypical habitat with *C. dissectum* found in a very different community type (Chapter 3). The population at Braunton Burrows (EBB) was highly genetically differentiated from the nearby populations, possibly through local adaptation, so its conservation should be a priority (Chapter 5).

6.2.2 Site management

Most sites within the British Isles were managed through grazing and sometimes occasional burning to reduce the tussocks of *Molinia caerulea* (Chapter 3). van Soest (2001) found that grazing pressure was the most important factor in determining species composition on the Culm grasslands of Devon. To conserve *C. dissectum*, grazing levels need to be sufficient to allow the creation of some bare ground to allow seedling establishment but should not be so high as to suppress flowering completely. It is important to note that abundant flowering does not necessarily equate to a healthy population if seedling recruitment is prevented.

Soil phosphorus levels (7 to 14 cm depth) are also of key importance. Levels are typically low in *C. dissectum* sites (0.5 to 5.1 mg kg^{-1}) but extremely low values (possibly through very intensive grazing) may be detrimental to *C. dissectum*, as they are related to low levels of allelic richness, increased inbreeding and lower germination under standard conditions (Chapter 4). However, if phosphorus levels become too high, *C. dissectum* will be out-competed by species that are able to build up biomass more quickly (Jongejans *et al.* 2006a). The effect of burning on *C. dissectum* was not investigated in this study although is an interesting area of further work. After burning at Knowstone Moor (EKS), *C. dissectum* appeared to have a positive response, growing well on the burnt tussocks of *Molinia caerulea* and rush species and this is likely to be an important mechanism for reducing biomass levels (**Plate 6.1**).

Plate 6.1 Regeneration of *Cirsium dissectum* after burning at Knowstone Moor.

6.2.3 Habitat restoration and re-creation

Topsoil removal to decrease soil fertility in areas with a suitable hydrological regime has been shown to be effective in the restoration of Cirsio-Molinietum grasslands in the Netherlands (Berendse *et al.* 1992; Jansen & Roelofs 1996; Jansen *et al.* 1996; Beltman *et al.* 2001) and research suggests that this method is also suitable for the British Isles (Tallowin & Smith 2001). In the Netherlands, restoration sites are often established next to existing Cirsio-Molinietum and natural colonisation allowed to take place as the bare soil created through the restoration process allows some successful establishment of *C. dissectum* to occur (Jongejans 2004). In this situation, where the size of the available habitat is being increased to allow population expansion, natural colonisation would seem to be the best policy (Gilbert & Anderson 1998).

In addition to increasing the extent of existing grasslands, it is also desirable to create or restore grasslands away from existing sites in order to create a habitat mosaic. In these situations, plant material has to be brought in from other sites and the type and source of material needs to be considered (Gilbert & Anderson 1998).

Seedling establishment in *C. dissectum* is very low in natural conditions but the disturbance created through the restoration process will allow some establishment to occur (Isselstein *et al.* 2002; Jongejans 2004). The low level of establishment means that the

number of individuals founding a new population will be small and Smulders *et al.* (2000) has shown that such populations have less genetic diversity and are differentiated from the donor population. Chapter 4 has shown that small populations have lower genetic diversity and that this relates to plant fitness, so restoration projects should use a large number of seeds to allow the establishment of as large a population as possible. An alternative is to grow seeds in more favourable conditions and use plug plants at restoration sites (Gilbert & Anderson 1998). Potential difficulties are that this removes an important selection phase within the life-cycle and would be considerably more labour intensive compared to sowing seed.

Local provenance is often considered important in restoration projects. This study has shown high levels of genetic differentiation and strong geographical structuring between *C. dissectum*, along with some indication of outbreeding depression (Chapter 5). All of these results suggest that plant material should be sourced locally. Chapter 4 showed, however, significant, positive relationships between population size and genetic diversity and between genetic diversity and fitness. Chapter 5 showed that selfed plants were subject to early acting inbreeding depression. Small, single populations of *C. dissectum* should therefore not be used as donor sites for restoration projects, as this is likely to lead to reduced genetic diversity, inbreeding depression and lower plant fitness. Ultimately, these factors may lead to the extinction of the restored population. The best approach would be to collect seed from several populations close to the restoration site, as this helps to increase levels of genetic diversity and also reduces collection pressure on existing populations. The genetic differentiation in Braunton Burrows (EBB) indicates, however, that all seed should be collected from the same habitat.

If sufficient seeds can be collected from nearby populations, then seeds should be the source of material used, as this equates best with natural colonisation processes. To further reduce founder effects, seeds could be collected from donor sites and sown on the

restoration site over a number of years. However, if it is not possible to collect sufficient seed, then it is recommended to grow seeds under glasshouse conditions and use plug plants for restoration.

6.3 Further work

This study has largely taken an observational approach to understanding the ecology and genetics of *C. dissectum*, as it was considered important to gain an understanding of what was happening in natural populations. This has revealed many relationships and hypotheses that can be tested experimentally. Bare soil and soil phosphorus levels have been shown to be important for population size, genetic diversity and fitness in *C. dissectum* and possible mechanisms for these relationships have been suggested. It would be valuable to test these hypotheses using a series of common garden and field experiments.

It was suggested that bare soil is important for the establishment of seedlings and clonal offspring and this could be tested by experimentally creating establishment gaps in natural populations. Safe sites for seedlings often require a number of factors in addition to bare soil and the requirements of safe sites could be investigated (Dinsdale *et al.* 2000). It would also be interesting to investigate the effects of phosphorus in field conditions, by fertilising plots with phosphorus at a number of field sites. There are many habitat factors that are also likely to be important for the ecology and genetics of *C. dissectum* that have not been investigated in this study. Key factors that warrant investigation under field conditions are the effect of hydrology, grazing and burning on species ecology.

The crossing experiment investigating the effects of inbreeding and outbreeding (Chapter 5) is another area for further investigation. The results showed marginally

significant relationships between the number and proportion of hard seeds produced per capitulum and the genetic distance between the individuals crossed. Further crosses could be performed to investigate whether greater sample sizes confirm or remove this relationship. The effects of selfing plants also requires a greater sample size; the genetic analysis suggested that *C. dissectum* had a mixed mating system but the number of seeds produced when individuals were crossed was very low. Selfing more plants would help to clarify these results.

The genetic analysis revealed intriguing results for Braunton Burrows (EBB) with higher genetic differentiation and lower genetic and genotypic diversity than expected. A reason for this may be strong selection pressures acting on this population and it would be possible to investigate this using reciprocal transplant experiments between EBB and a nearby rhos pasture site. The pattern of genetic diversity seen may also be due to founder effects. Throughout the EBB site were a number of smaller *C. dissectum* populations in addition to the one sampled. It is possible that these act as meta-populations with frequent extinction and colonisation events occuring. Observing the demography of plants throughout Braunton Burrows over a number of years would allow investigation of this idea.

The most intriguing area for further work would be to investigate hybridization between *Cirsium* species in the British Isles. *C.* x *forsteri* is the commonest hybrid thistle; it was found in two of the populations surveyed for this study and can be found wherever the two parents are present (Preston *et al.* 2002). It would be interesting to investigate the effects of hybridization on the ecology of the parents and hybrids and also to extend this to other *Cirsium* species (the pattern of hybridization is shown in **Table 6.1**). Key questions are what factors allow hybridization to occur and are these constant throughout the range of the hybrid and what is the effect of hybridization on the fitness of the hybrids and parents?

The first stage would be to carry out crosses between different *Cirsium* species to assess the degree of fertility of F1 hybrids and backcrosses. The biology of the plants could be investigated to assess what pre- and post-zygotic mechanisms are in place to prevent hybridization (Wolf *et al.* 2001). The relative fitness of the parents and hybrids could then be investigated in standard conditions and within the plants habitat to see whether hybrids are likely to out-compete parents. Experimental populations could be established to investigate whether hybridization is more likely when one species has a much smaller population size than the other and to test other factors that may increase levels of hybridization. This could be supplemented by examining natural levels of hybridization in a number of populations and relating this to the abundance of the parents along with other factors. *Cirsium* hybrids can often be recognised morphologically but genetic markers would need to be used to check how accurate morphological identification was and also to identify backcrossed individuals that are more likely to look like the parent plants. These questions have wide scientific relevance, as hybridization can potentially lead to the extinction of rare species, or have positive effects through the introduction of novel alleles; it can also lead to speciation events (Rieseberg 1991; Wolf *et al.* 2001). The high level of natural hybridization in *Cirsium* and the fact that some species have a very limited distribution and specialised habitat requirements, whilst others are widespread and weedy, makes this genus an excellent study system to investigate the effects of hybridization.

Table 6.1 Hybridization in *Cirsium* species in the British Isles (Stace 1997). (Parents are listed at the top and left of the matrix and the names of the hybrids formed shown. The number in parentheses is the number of hybrids formed by that species).

Cirsium	*vulgare*	*tuberosum*	*palustre*	*heterophyllum*	*eriophorum*	*dissectum*	*arvense*	*acaule*
***acaule*(5)**	*sabaudum*	*medium*	*kirschlegeri*			*woodwardii*	*boulayi*	
***arvense*(2)**			*celakovskianum*					
***dissectum*(2)**			*forsteri*					
***eriophorum*(1)**	*grandiflorum*							
***heterophyllum*(1)**			*wankelii*					
***palustre*(6)**	*subspinuligerum*	*semidecurrens*						
***tuberosum*(2)**								
***vulgare*(3)**								

6.4 Conclusion

C. dissectum is an Oceanic Temperate species found in nutrient deficient grasslands in north-west Europe. It has declined throughout its range, due to changes in agriculture, and its long-term survival is dependent on suitable site management and habitat restoration. Management should ensure that habitat heterogeneity is maintained, so that flowering can occur but bare soil is available for seedling establishment. Habitat restoration should use large numbers of seeds collected from a number of local populations. As an indicator species of Cirsio-Molinietum and a representative of semi-natural, nutrient-poor grasslands these suggestions are also likely to be applicable to other species found within these habitats.

Appendix A

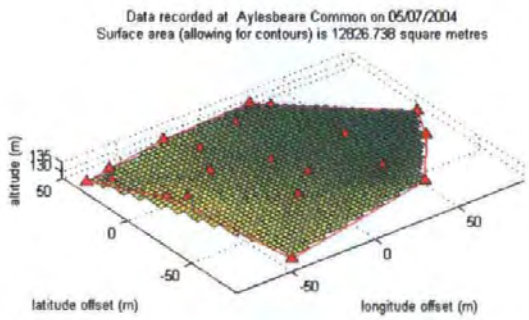
Site descriptions

Name	Aylesbeare Common
Code	EAC
Country	England
Latitude	N50.70671
Longitude	W3.34167
Grid reference	SY052910

Description 24/06/03

Heathland containing a mosaic of habitats. *C. dissectum* was found in a few lower lying areas. The site sampled contained the largest population of *C. dissectum,* which was found in heathy vegetation composed of *Ulex gallii, Calluna vulgaris, Erica tetralix* and *E. cinerea* separated by closely grazed cattle tracks that contained a short sward and bare soil caused by poaching. Electric fencing loops were used to concentrate grazing on particular areas with the site surveyed grazed during the summer months.

Population topography



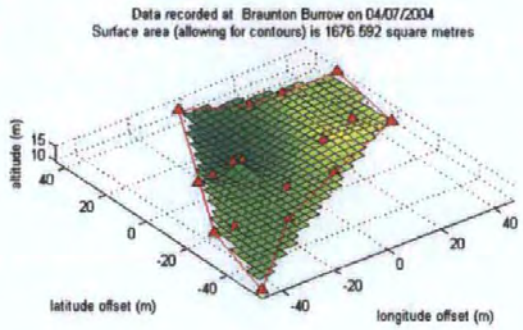
Left: site overview 09/08/02. Right: Close up of rosette in 50cm^2 quadrat showing bare soil

Name	Braunton Burrows
Code	EBB
Country	England
Latitude	N51.09103
Longitude	W4.20039
Grid reference	SS459351

Description 21/06/03

C. dissectum is found in a few locations throughout Braunton Burrows, with the site surveyed containing the largest population. It is a dune slack that becomes quite dry in the summer months and often has standing water in winter. The site is cut approximately every two years.

Population topography



Left: Site overview 21/06/03. Right: winter, showing standing water

Name	Baddesley Common
Code	EBC
Country	England
Latitude	N50.98966
Longitude	W1.44360
Grid reference	SU390217

Description 15/06/03

A mosaic of fields and open areas containing a wide range of habitats. *C. dissectum* was found growing in a very closely grazed pasture, where small, low-growing vegetative rosettes were present but no flowering rosettes. The site was grazed by ponies.

Population topography: Map not available

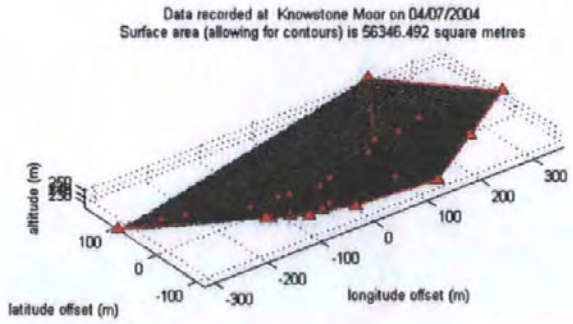
Top: view of site 15/06/03. Bottom: close up of rosettes 03/08/03

Name	Knowstone Moor
Code	EKS
Country	England
Latitude	N50.97763
Longitude	W3.64604
Grid reference	SS84442157

Description 21/06/03

A large rhos pasture with *C. dissectum* found in a long strip in a more low-lying area. The site was grazed with cattle in the summer months and sometimes burnt.

Population topography



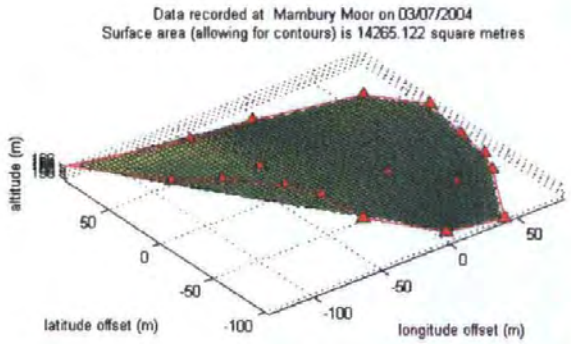
Left: site overview 21/06/03. Right: *C. dissectum* flowers 21/06/03

Name	Mambury Moor
Code	EMM
Country	England
Latitude	N50.53'8
Longitude	W4.17'9
Grid reference	SS385171

Description 11/05/03

Large rhos pasture with abundant *C. dissectum*. The sward was tall and interspersed with clumps of willow. The site margins were wetter and appeared to contain species typical of more productive sites, suggesting possible run-off from the surrounding improved pastures. Cattle grazing occured during the summer months.

Population topography



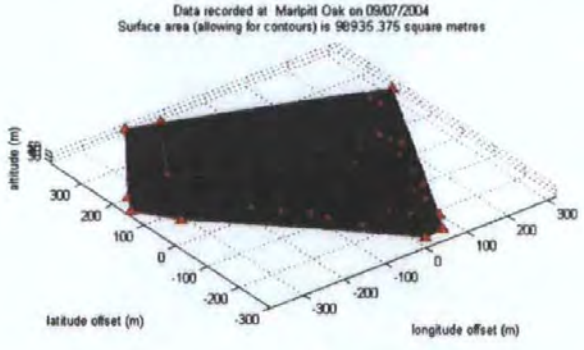
Site overview 11/05/03

Name	Marlpitt Oak
Code	EMO
Country	England
Latitude	N50.79641
Longitude	W1.59453
Grid reference	SU285002

Description 14/06/03

A large open heath within the New Forest where a very closely grazed sward was interspersed with *Ulex minor, Calluna vulgaris, Erica tetralix* and *Erica cinerea* and some *Myrica gale. C. dissectum* occured as small, low growing rosettes in the short sward, with flowering rosettes confined to the edges of the taller growing gorse, where it was presumably protected from grazing. There were abundant areas of bare soil and the site was grazed all year by ponies and cattle.

Population topography



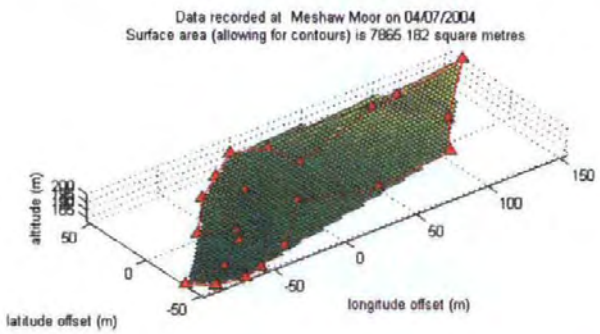
Left: site overview 14/06/03. Right: close up of vegetative rosettes 11/08/02

Name	Meshaw Moor
Code	EMS
Country	England
Latitude	N50.95052
Longitude	W3.76680
Grid reference	SS758187

Description 22/06/03

Meshaw Moor was composed of a number of small fields separated by wooded boundaries. *C. dissectum* was found within two of the fields, although only a small amount was present in one of these. The site sampled was more abundant in *C. dissectum*, although its presence was fairly patchy. The site was heterogeneous, with short swards interspersed with more overgrown areas, containing abundant *C. palustre*. It was cattle-grazed during the summer.

Population topography



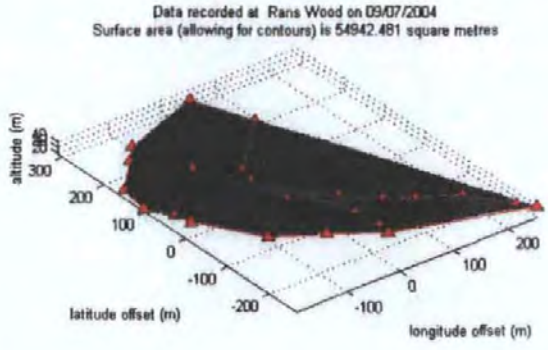
Left: Site overview 22/06/03. Right: Close up of *C. dissectum* in vegetation 22/06/03

Name	Rans Wood
Code	ERW
Country	England
Latitude	N50.32223
Longitude	W1.48491
Grid reference	SU362031

Description 14/06/03

A large heterogeneous heath within the New Forest with dry heath interspersed with wet, bog areas. *C. dissectum* was found in an area of moist heath containing a short sward and occasional *Myrica gale* plants. The short sward contained small, low-growing rosettes whilst flowering rosettes tended to be found in the cover of *Myrica gale* and other taller species. The site was grazed all year by ponies and cattle.

Population topography



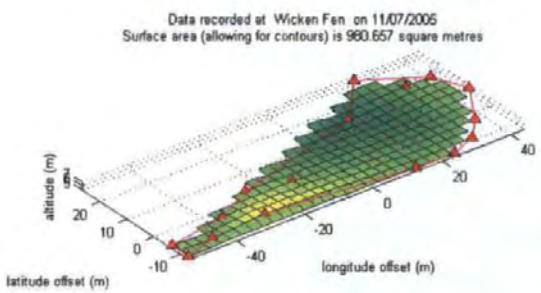
Left: site overview 14/06/03. Right: Close up of *C. dissectum* in vegetation 14/06/03

Name	Wicken Fen
Code	EWF
Country	England
Latitude	N52.186
Longitude	E0.175
Grid reference	TL562705

Description 26/04/03

C. dissectum may have occured in small patches throughout Wicken Fen but the site surveyed was the only one that could be found and was the smallest out of all the 22 populations that were surveyed. It occurred in a field that was cut for litter. *C.* x *forsteri* was also observed at the site.

Population topography



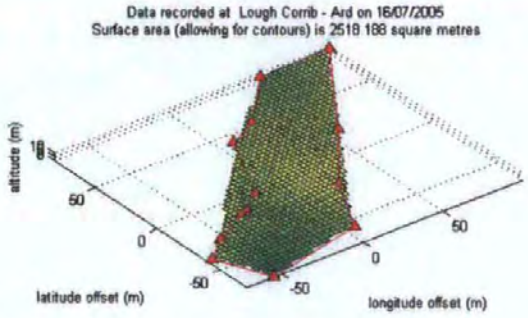
Left: site overview 26/04/03. Right: site overview 28/07/03

Name	Lough Corrib
Code	IAR
Country	Ireland
Latitude	N53.42866
Longitude	W9.24855
Grid reference	M170434

Description 29/05/03

Schoenus nigricans fen on the shores of Lough Corrib. There was no evidence of any management at this site and the vegetation was tall, with a considerable litter layer. There were large tussocks of *S. nigricans* and *Molinia caerulea* with *C. dissectum* interspersed throughout. The *C. dissectum* leaves were very large and the flowering stems tall enough to overtop the average vegetation height.

Population topography



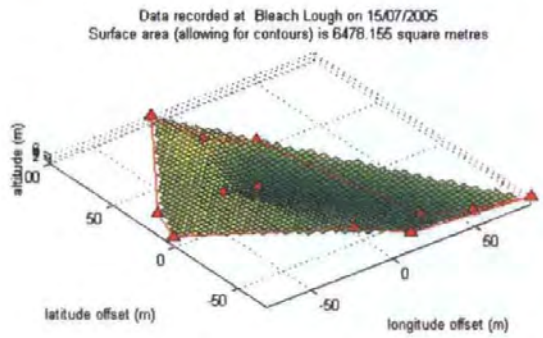
Left: site overview 29/05/03. Right: *C. dissectum* within vegetation 29/05/03

Name	Bleach Lough
Code	IBL
Country	Ireland
Latitude	N52.64435
Longitude	W8.82558
Grid reference	R441557

Description 01/06/03

Pasture on the edge of Bleach Lough containing *Schoenus nigricans*, along with a number of rush species and abundant *Cirsium palustre*. The vegetation was more open in some areas with moss-covered hummocks. The site was grazed by cattle in the summer.

Population topography



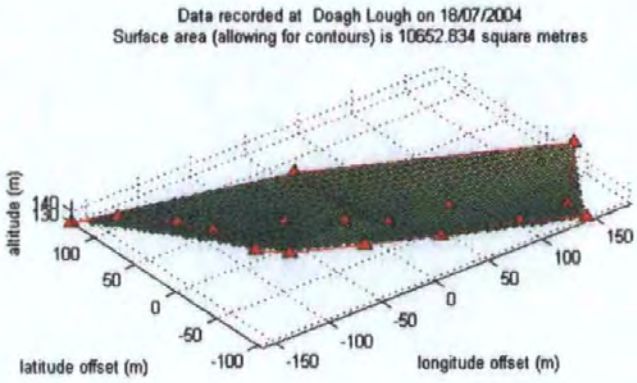
Overview of site: 01/06/03

Name	Doagh Lough
Code	IDL
Country	Ireland
Latitude	N54.41671
Longitude	W7.87734
Grid reference	H079526

Description 25/05/03

Pasture on the shores of Doagh Lough where *Molinia caerulea* was dominant, c. 20 bullocks grazing on the date of sampling 25/5/03. The pasture sloped quite steeply away from the shore resulting in some areas with very high levels of poaching.

Population topography



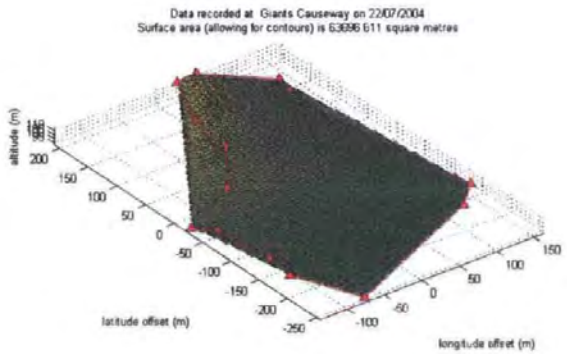
Left: overview of site, edge of lough 25/05/03. Right: overview of site, sloping pasture 25/05/03.

Name	Giant's Causeway
Code	IGC
Country	Ireland
Latitude	55.24495N
Longitude	6.49889W
Grid reference	C944445

Description 23/05/03

A large field separated from the sea by a coastal path. Surrounding fields appeared more improved and contained sheep. This field had evidence of cattle grazing and showed some poaching but no cattle were seen at the time of the survey. Short sward height with many large patches of *C. dissectum.*

Population topography



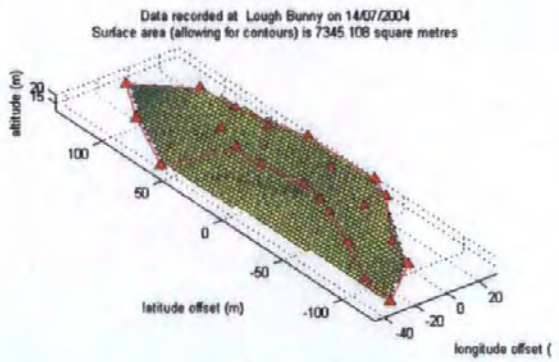
Left: overview of site, *C. dissectum* was found in the field beyond the fence 23/05/03. Right: *C. dissectum* rosettes within the vegetation.

Name	Lough Bunny
Code	ILB
Country	Ireland
Latitude	N53.02300
Longitude	W8.92018
Grid reference	R382979

Description 30/05/03

Schoenus nigricans fen on the shores of Lough Bunny. Tall vegetation was interspersed with areas of limestone pavement. Management unknown.

Population topography



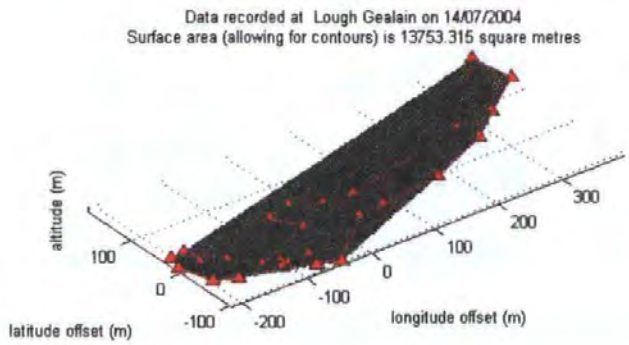
Left: position of site in relation to Lough Bunny in the background. Right: site overview 30/05/03

Name	Lough Gealain
Code	ILG
Country	Ireland
Latitude	N52.99925
Longitude	W9.01795
Grid reference	R316954

Description 30/05/03

An area of *Schoenus nigricans* fen extending around the shores of Lough Gealain. *C. dissectum* was found near the margin of the Lough and extended into the moist area surrounding it on to the edge of the limestone pavement. Some *C. dissectum* rosettes were flowering within the crevices of the limestone. No cattle were seen on the day of surveying but old cowpats were found.

Population topography



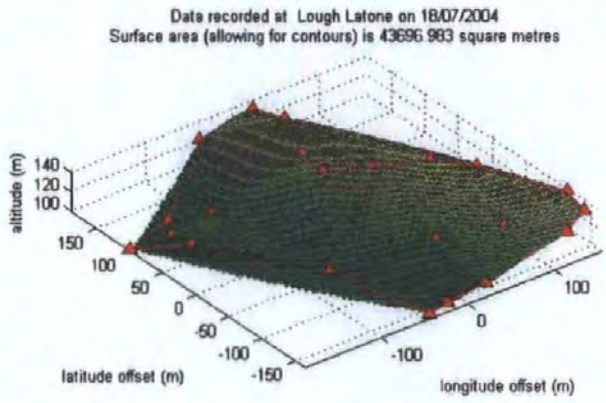
Left: overview of site. Right: *C. dissectum* within the vegetation 30/05/03

Name	Lough Lattone
Code	ILL
Country	Ireland
Latitude	N54.36579
Longitude	W7.98614
Grid reference	H009470

Description 26/05/03

Large area of pasture with a very short sward. The site sloped steeply and showed considerable cattle poaching. Cattle were present on the sampling date 26/05/03. *C. dissectum* was relatively abundant throughout the site but nearly all of the rosettes were vegetative.

Population topography



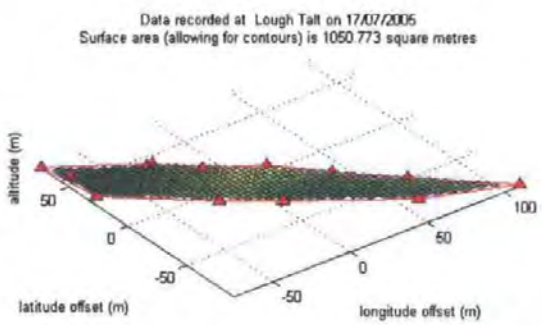
Left: overview of site 26/05/03. Right: close up of vegetation showing cattle poaching 26/05/03

Name	Lough Talt
Code	ILT
Country	Ireland
Latitude	N54.08472
Longitude	W8.92027
Grid reference	G397161

Description 27/05/03

Rough ground on the shores of Lough Talt with a mosaic of vegetation types. Large patches of *C. dissectum* were found in areas with *Molinia caerulea* cover but where the height of the grass was very high no *C. dissectum* was found. Areas close to the shore had abundant bare soil and some *C. dissectum* was found there. No management had taken place for a number of years but the site had been cut for silage in the past and some of the older gorse seemed to have been burnt.

Population topography



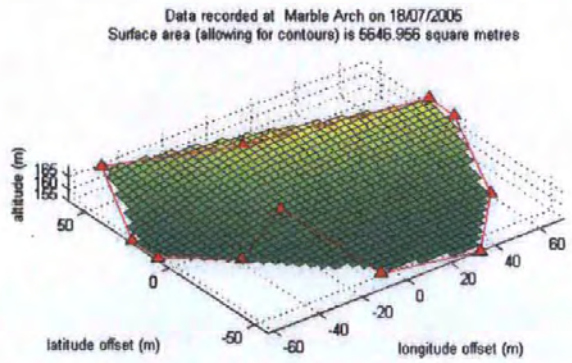
Left: site overview 27/05/03. Right: *C. dissectum* rosettes in area with short vegetation 27/05/03.

Name	Marble Arch
Code	IMA
Country	Ireland
Latitude	N54.25955
Longitude	W7.81974
Grid reference	H117352

Description 26/05/03

A sloping grassland in a limestone area, a small population of *C. dissectum* was found growing either side of a track that lead down to a large field containing cattle.

Population topography



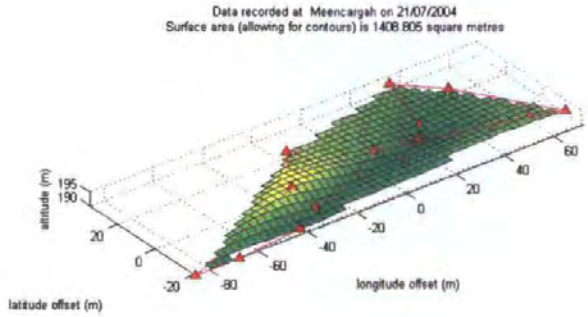
Site overview, *C. dissectum* was found on either side of the track 26/05/03.

Name	Meencargagh
Code	IME
Country	Ireland
Latitude	N34.64700
Longitude	W7.55232
Grid reference	H289784

Description 24/05/03

An area of rough grassland with large *Molinia caerulea* tussocks, about 300 m from the road. *C. dissectum* was found in large leafy patches on either site of a small waterway for a length of c. 195 m. After this the water ran through a fenced off area of improved grassland, where *C. dissectum* was not found. The site was unfenced and ran into an area of open moorland. No evidence of management.

Population topography



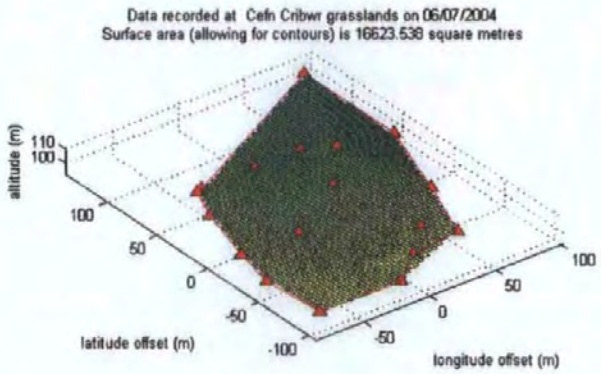
Site overview 24/05/03

Name	Cefn Cribwr
Code	WCG
Country	Wales
Latitude	N51.53534
Longitude	W3.63463
Grid reference	SS866835

Description 30/06/03

A series of fields at least two of which contained *C. dissectum.* The site surveyed was a typical rhos pasture with fairly open vegetation interspersed with some more overgrown patches. Site was grazed by cattle during the summer.

Population topography



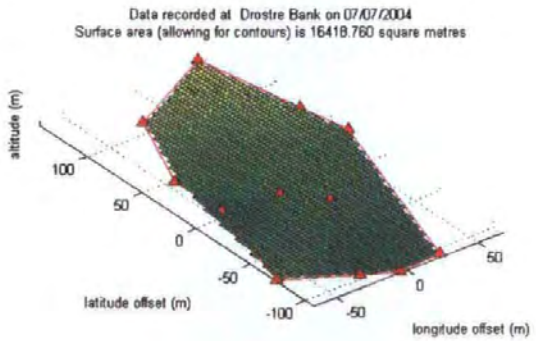
Two overviews of site 30/06/03

Name	Drostre Bank
Code	WDB
Country	Wales
Latitude	N51.97392
Longitude	W3.31493
Grid reference	SO096318

Description 29/06/03

Rhos pasture surrounded by improved fields and an area of woodland. Some areas appeared quite productive, whilst others were more open. The site was grazed with cattle during the summer. *C. dissectum* was abundant throughout most of the site and flowered freely.

Population topography



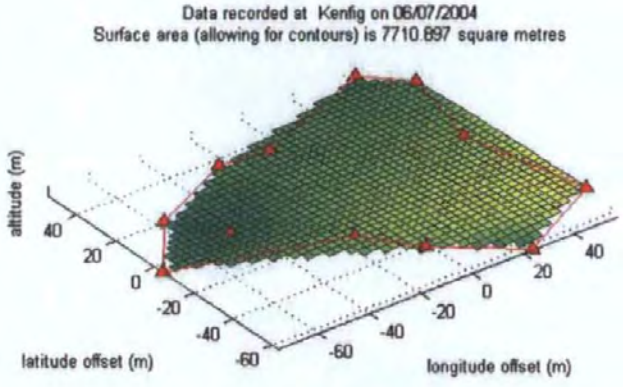
Left: site overview 29/06/03 Right: *C. dissectum* in flower 29/06/03

Name	Kenfig
Code	WKF
Country	Wales
Latitude	N51.51681
Longitude	W3.75215
Grid reference	SS78428169

Description 07/06/03

Cirsium dissectum was found in dune slacks and other moist areas throughout Kenfig. It was very patchy with individual patches being very large in places. The proportion of rosettes flowering appeared greater here than at other sites. The site sampled was the largest continuous population, lying along a dune slack. *C.* x *forsteri* was found flowering within the population sampled. The site was sometimes cut.

Population topography



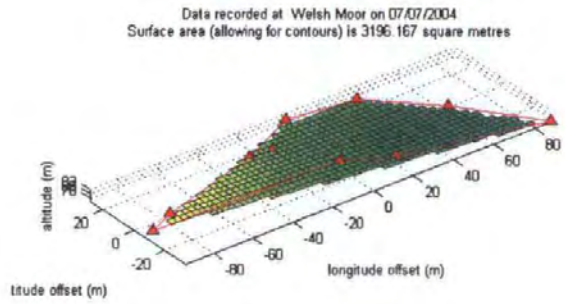
Left: site overview 07/06/03 Right: flowering rosettes 07/06/03

Name	Welsh Moor
Code	WWM
Country	Wales
Latitude	N51.61389
Longitude	W4.14153
Grid reference	SS517932

Description 30/06/03

Area of rhos pasture within a moorland mosaic. The site surveyed was small but quite heterogeneous with short swards, areas with tussocky *Molinia caerulea* and areas with abundant rushes. *C. dissectum* grew along a linear area, possibly indicating the influence of a base-rich flush. The site was grazed with cattle during the summer and sometimes burnt.

Population topography



Date of photo:

Appendix B

Soil nutrients and vegetation structure

Table B-1 Soil nutrients and vegetation structure of 22 sites containing *Cirsium dissectum*. (Sites for comparison indicated with * are from Goodwin (1995). Summer grazing was defined as sites grazed for less than six months and continuous grazing as sites grazed for six months or more).

	Management	Bare soil % (SD)	Vegn. height mm (SD)	pH (0-14cm)	OM % (0-14cm)	Total N % (0-14cm)	P mg kg^{-1} (7-14cm)	P mg kg^{-1} (0-7cm)	K mg kg^{-1} (0-14cm)	Ca mg kg^{-1} (0-14cm)
EAC	summer grazing	15 (19)	440 (400)	4.7	12	0.3	0.7	0.0	113	331
EBB	Cut	5 (2)	205 (109)	5.9	14	0.2	0.6	1.1	50	6236
EKS	summer grazing	1 (3)	320 (84)	4.9	15	0.4	0.7	0.0	72	252
EMM	summer grazing	0 (0)	499 (135)	4.6	23	0.5	1.1	4.9	140	248
EMO	continuous grazing	20 (13)	149 (110)	4.7	10	0.2	0.5	11.7	52	459
EMS	summer grazing	7 (7)	515 (71)	4.8	19	0.5	1.9	2.1	181	413
ERW	continuous grazing	20 (20)	287 (92)	4.5	16	0.5	1.1	6.4	122	390
EWF	Cut	0 (0)	833 (238)	5.0	82	2.4	1.7	8.6	75	10292
IAR	None	0 (0)	596 (114)	4.9	87	1.7	5.1	10.5	94	4177
IBL	summer grazing	6 (6)	405 (99)	5.8	73	1.6	3.2	2.5	160	12112
IDL	continuous grazing	20 (17)	226 (116)	5.4	12	0.3	0.5	0.0	70	1345
IGC	?	3 (3)	173 (115)	5.0	36	0.5	0.8	0.1	529	998
ILB	?	6 (11)	419 (52)	6.1	34	0.8	2.0	3.6	55	6225
ILG	?	3 (4)	424 (72)	6.1	58	1.2	1.8	3.3	39	9482
ILL	continuous grazing	17 (15)	107 (58)	4.9	13	0.4	0.9	1.4	54	973
ILT	none/cut/burnt	12 (25)	388 (155)	6.0	8	0.1	0.9	1.3	18	4089
IMA	?	6 (8)	116 (18)	5.4	11	0.2	1.1	0.0	59	1433
IME	None	0 (0)	599 (181)	4.6	79	1.9	1.1	2.2	201	2208
WCG	summer grazing	6 (7)	371 (161)	4.7	25	0.6	1.6	0.8	118	414
WDB	summer grazing	1 (6)	346 (125)	5.0	29	0.8	2.2	12.4	237	3212
WKF	Cut	8 (9)	205 (146)	6.0	6	0.1	0.7	0.0	27	4008
WWM	summer grazing	1 (2)	392 (160)	5.2	19	0.4	1.2	8.3	118	781
Mean (SD)		**7 (7)**	**364 (179)**	**5.2 (0.6)**	**31 (27)**	**0.7 (0.6)**	**1.4 (1.1)**	**3.7 (4.1)**	**117 (109)**	**3185 (3583)**
Acid grassland (*Festuca-Agrostis* Upland)*				**5.2**			**1.3**		**112**	**395**
Neutral grassland (Neutral *Molinia*)*				**6.1**			**27.0**		**240**	**7530**
Calcareous grassland (chalk grassland)*				**7.6**			**7.0**		**150**	**11000**
Improved grassland on Culm measures*				**5.7**			**14.0**		**250**	**no data**

Appendix C

Plant communities

Table C-1 Plant species found in 11 sites in Britain where *Cirsium dissectum* was present. (Ten 2 x 2 m quadrats were surveyed at each site; in each quadrat all species were identified and abundance estimated using the Domin scale. Roman numerals indicate species frequency (the number of quadrats a species occured within): I 1 to 20%; II 21 to 40%; III 41 to 60%; IV 61 to 80% and V 81 to 100%. The numbers in parentheses are the Domin range across the quadrats. Sites were assigned to National Vegetation Classification (NVC) communities using MAVIS Plot Analyser v. 1 (Smart 2000) and Rodwell (1991, 1995, 2000)).

Site Code	**EBC**	**EKS**	**EMS**	**EMM**	**WDB**	**EAC**	**EMO**	**ERW**	**EBB**	**WKF**	**EWF**
NVC Community	**M24c**	**M24c**	**M24c**	**M24c**	**M24**	**M16b**	**M16b**	**M16b**	**SD14b**	**SD14d**	**S24c**
Similarity (from MAVIS)	**51.16**	**57.55**	**52.75**	**53.07**	**56.96**	**57.37**	**52.46**	**53.47**	**47.24**	**54.23**	**58.57**
Number of species	**40**	**34**	**32**	**28**	**44**	**18**	**27**	**25**	**29**	**41**	**26**
Achillea ptarmica	V (1-4)	I (1)	IV (1)	II (1)	-	-	-	-	-	-	-
Agrostis canina canina	V (5-9)	V (2-8)	V (3-6)	V (4-6)	I (1-4)	-	IV (1-2)	V (4-8)	-	-	-
Agrostis capillaris	III (2-8)	-	-	-	-	-	-	II (1-4)	-	-	-
Agrostis curtisii	-	-	-	-	-	III (2-8)	V (1-7)	IV (4-5)	-	-	-
Agrostis gigantea	-	-	II (1-8)	-	-	-	-	-	-	-	-
Agrostis stolonifera	II (2-6)	-	-	-	III (1-7)	-	-	-	V (8-9)	IV (1-4)	III (4-7)
Anagallis tenella	-	I (1)	-	-	I (1)	-	I (1)	-	-	III (1-3)	-
Angelica sylvestris	-	-	-	-	I (1)	-	-	-	-	-	IV (1-6)
Anthoxanthum odoratum	I (1)	III (1-3)	III (1)	III (1-2)	IV (1-6)	-	-	-	-	I (1)	-
Asperula cynanchica	II (1-4)	-	-	-	V (1-4)	-	I (1)	I (1)	IV (1)	IV (1-3)	-
Betula pendula seedling	III (1)	-	-	-	-	-	-	II (1)	-	-	-
Betula pubescens seedling	-	-	-	-	-	I (1)	I (1)	-	-	-	-
Briza media	-	-	-	-	I (1)	-	-	-	-	IV (1-8)	-
Calluna vulgaris	I (1)	I (1)	I (2)	III (2-5)	-	V (4-7)	V (2-6)	IV (1-5)	-	-	-
Calystegia sepium	-	-	-	-	-	-	-	-	-	-	II (1)
Carex viridula oedocarpa	-	-	I (1)	IV (1)	-	II (3-4)	V (1-5)	V (1-7)	-	-	-
Carex echinata	-	V (1-3)	I (1)	-	-	-	-	-	-	-	-
Carex flacca	-	-	II (1-5)	I (1-2)	III (1)	-	-	-	-	I (1)	-
Carex hostiana	-	-	-	IV (1-5)	IV (1-4)	-	-	-	-	-	-
Carex nigra	I (1)	-	-	-	II (1)	-	-	-	V (2-8)	IV (1-8)	-
Carex ovalis	III (1-5)	-	-	-	-	-	-	-	-	-	-
Carex panicea	III (1-5)	V (3-5)	IV (1-5)	V (4-7)	IV (1-5)	IV (1-5)	V (3-7)	V (1-6)	III (2-3)	V (1-6)	IV (1-8)
Carex pulicaris	-	I (1)	-	IV (1)	IV (1-3)	I (1)	I (1)	-	-	-	-
Centaurea nigra	-	-	-	-	II (1)	-	-	-	-	I (1)	-
Cirsium dissectum	IV (4-9)	V (1-6)	V (1-6)	V (3-8)	V (1-8)	IV (1-4)	V (3-5)	V (2-6)	III (3-9)	V (3-8)	II (1-5)
Cirsium palustre	II (1-4)	II (1-5)	V (1-5)	I (1)	IV (1)	-	-	-	-	I (4)	III (1-5)
Cynosurus cristatus	-	-	-	-	II (1)	-	-	-	-	-	-
Dactylorhiza maculata	-	IV (1)	-	V (1)	II (1)	-	-	-	-	-	-
Dactylorhiza sp.	-	-	-	-	-	-	-	-	I (1)	II (1)	-
Danthonia decumbens	I (2)	IV (1-3)	-	V (1-3)	III (1-5)	-	V (1-3)	V (1-4)	-	I (1-3)	-
Deschampsia cespitosa	-	-	-	-	-	-	-	-	-	-	I (4)
Eleocharis palustris	-	-	-	-	-	-	-	-	-	I (9)	-
Epilobium hirsutum	-	-	-	-	-	-	-	-	-	-	I (1)
Epilobium palustre	I (1)	I (1)	I (1)	-	II (1)	-	-	-	-	-	-
Epipactis palustris	-	-	-	-	-	-	-	-	IV (1-3)	V (1-3)	-
Equisetum palustre	-	-	-	-	II (1)	-	-	-	V (1-3)	IV (1-4)	-
Erica cinerea	-	-	-	-	-	II (1-5)	III (1-4)	I (1)	-	-	-
Erica tetralix	I (1)	V (4-6)	-	IV (4-6)	-	V (4-7)	V (1-7)	II (1-4)	-	-	-
Eriophorum angustifolium	-	-	-	-	-	-	-	-	-	I (1)	-
Euphrasia nemorosa	-	-	-	-	-	-	-	-	-	I (1)	-
Festuca rubra	-	IV (4-7)	-	-	V (1-8)	-	-	-	-	-	-
Filipendula ulmaria	-	-	-	-	II (1-4)	-	-	-	I (1)	-	V (1-7)

Fragaria vesca	-	-	-	-	-	-	-	-	-	I (1)	-
Fraxinus excelsior seedling	-	-	-	-	I (1)	-	-	-	-	-	-
Galium uliginosium	-	-	-	-	-	-	-	-	-	-	III (1-4)
Holcus lanatus	IV (1-8)	IV (1-4)	III (1-2)	II (1-3)	V (1-6)	-	-	-	I (1)	III (1-3)	I (1)
Hydrocotyle vulgaris	I (4)	II (1-3)	-	-	-	-	-	I (3)	V (1-9)	V (4-8)	III (3-7)
Iris pseudoacorus	-	-	-	-	-	-	-	-	-	-	IV (1-2)
Juncus acutiflorus	IV (1-7)	V (1-8)	IV (1-4)	III (1-8)	V (1-8)	II (1-3)	-	II (1-6)	-	III (1-4)	-
Juncus articulatus	-	-	-	-	-	-	-	-	II (1)	-	-
Juncus conglomeratus	-	III (1-5)	III (1-7)	IV (1-5)	III (1-8)	III (2-8)	-	-	-	-	-
Juncus effusus	I (1)	I (1)	V (1-7)	I (5-7)	-	II (1)	-	-	-	-	-
Juncus squarrosus	-	-	-	-	-	-	III (1-4)	-	-	-	-
Juncus subnodulosus	-	-	-	-	-	-	-	-	-	-	V (3-9)
Lactuca serriola	-	-	-	-	-	-	-	-	-	I (1)	-
Lathyrus palustris	-	-	-	-	-	-	-	-	-	-	I (1-3)
Lathyrus sp.	-	-	I (1)	-	-	-	I (1)	-	-	-	-
Leontodon autumnalis	-	-	-	-	-	-	-	-	-	II (1-2)	-
Leontodon hispidus	-	-	-	-	-	-	-	-	-	I (2)	-
Leontodon saxatilis	I (2)	-	-	-	-	-	-	-	-	-	-
Lotus corniculatus	-	-	-	-	-	-	-	-	IV (1-4)	IV (1-4)	-
Lotus pedunculatus	V (1-9)	II (1-4)	V (2-6)	IV (1-3)	V (1-4)	-	-	-	-	-	-
Luzula multiflora	I (4)	II (1-3)	I (1)	III (1)	IV (1-3)	-	-	I (1)	-	-	-
Lychnis flos-cuculi	-	-	-	-	III (1)	-	-	-	-	I (1)	I (1)
Lycopus europaeus	I (1)	-	-	-	-	-	-	-	-	I (1)	-
Lysimachia vulgaris	-	-	-	-	-	-	-	-	-	-	III (1-5)
Lythrum salicaria	-	-	-	-	-	-	-	-	-	-	III (1-4)
Melilotus officinalis	-	-	-	-	-	-	-	-	V (1-7)	-	-
Mentha aquatica	II (1-4)	-	-	-	-	-	-	-	V (1-5)	IV (1-4)	II (1-3)
Molinia caerulea	V (4-7)	V (9-9)	V (8-9)	V (7-9)	V (7-9)	V (6-9)	V (6-9)	V (5-8)	-	III (4-5)	IV (7-9)
Myrica gale	-	-	-	-	-	-	-	I (6)	-	-	-
Narthecium ossifragum	-	V (3-8)	-	-	-	-	-	-	-	-	-
Odontites vernus	-	-	-	-	-	-	-	-	I (1)	-	-
Oenanthe lachenalii	-	-	-	-	-	-	-	-	V (1-3)	-	-
Ophioglossum vulgatum	-	-	-	-	-	-	-	-	I (1)	-	-
Parentucellia viscosa	-	-	-	-	-	-	-	-	II (1-2)	-	-
Pedicularis palustris	I (1)	-	-	-	-	-	-	-	-	-	-
Pedicularis sylvatica	-	-	-	-	-	-	IV (1-3)	-	-	-	-
Phragmites australis	-	-	-	-	-	-	-	-	-	-	V (1-7)
Plantago lanceolata	-	-	III (1-3)	-	I (1)	-	-	-	I (1)	III (1-7)	-
Plantago major	I (1)	-	-	-	-	-	-	-	-	-	-
Polygala serpyllifolia	-	I (1)	-	-	-	-	III (1)	-	-	-	-
Polygala vulgaris	-	-	-	-	-	-	-	-	-	I (1)	-
Polytrichum sp.	-	-	I (1)	-	-	-	-	-	-	-	-
Potentilla anserina	-	-	-	-	-	-	-	-	V (6-9)	II (3-5)	-
Potentilla erecta	IV (1-5)	V (2-4)	V (1-4)	V (2-4)	IV (1-4)	V (1-4)	V (1-4)	V (1-4)	-	-	-
Potentilla reptans	-	-	-	-	-	-	-	-	V (1-8)	-	-
Prunella vulgaris	II (1-4)	I (1)	I (1)	-	II (1)	-	-	I (4)	-	I (1)	-
Pteridium aquilinum	-	-	-	-	-	I (7)	-	I (1)	-	-	-
Pulicaria dysenterica	-	-	-	-	-	-	-	-	III (1)	V (1-8)	-
Pyrola rotundifolia	-	-	-	-	-	-	-	-	-	I (1)	-
Quercus robur seedling	-	-	II (1)	-	I (1)	-	-	-	-	-	-
Ranunculus acris	-	I (1-2)	-	-	V (1)	-	-	-	V (1-3)	V (1-4)	-
Ranunculus flammula	IV (2-8)	-	I (1)	-	IV (1-4)	-	I (1)	-	V (1-3)	V (1-5)	-
Ranunculus repens	III (1-6)	-	III (1)	-	-	-	-	-	-	-	-
Rhinanthus minor	-	-	-	-	-	-	-	-	-	III (1-3)	-
Rubus caesius	-	-	-	-	-	-	-	-	-	I (1)	-

Rubus fruticosus agg.	I (1)	-	-	-	-	-	I (2)	I (1)	-	-	-
Rumex acetosa	-	I (1)	III (1-2)	-	III (1)	-	-	-	-	-	II (2)
Sagina sp.	-	-	-	-	-	-	I (1)	-	-	-	-
Salix repens	III (1)	-	-	I (1)	-	IV (1-4)	II (1-3)	V (1-5)	II (5-8)	V (1-7)	-
Scutellaria galericulata	-	-	-	-	-	-	-	-	-	I (1)	-
Scutellaria minor	II (1-2)	III (1-3)	I (1)	IV (1-3)	I (1)	-	-	-	-	-	-
Senecio jacobaea	I (1)	-	-	-	-	-	-	-	-	-	-
Serratula tinctoria	-	I (1-4)	III (1-2)	V (1-3)	-	-	II (1)	I (1)	-	-	-
Sphagnum sp.	-	V (8-8)	IV (3-5)	V (3-6)	I (6-8)	II (1-5)	-	-	-	-	-
Stachys palustris	-	-	-	-	-	-	-	-	-	-	II (1)
Succisa pratensis	II (1-7)	V (2-5)	V (1-6)	IV (2-6)	IV (1-5)	-	II (1)	IV (1-4)	-	-	I (1)
Symphytum officinale	-	-	-	-	-	-	-	-	-	-	IV (1-5)
Taraxacum officinale agg.	I (1)	-	-	-	I (1)	-	-	-	-	-	-
Thalictrum flavum	-	-	-	-	-	-	-	-	-	-	IV (1-5)
Trifolium dubium	-	-	-	-	-	-	-	-	-	I (1)	-
Trifolium fragiferum	-	-	-	-	-	-	-	-	IV (1-5)	III (1-5)	-
Trifolium pratense	-	-	-	-	I (1-4)	-	-	-	III (1-5)	V (1-5)	-
Trifolium repens	II (1-4)	-	-	-	II (1-5)	-	-	-	IV (1-5)	II (1)	-
Ulex gallii	-	-	-	II (4-6)	-	V (6-8)	-	-	-	-	-
Ulex minor	-	-	-	-	-	-	V (1-5)	I (1)	-	-	-
Veronica scutellata	I (1)	-	-	-	-	-	-	-	-	-	-
Viola canina	-	-	-	-	-	-	IV (1-2)	-	-	-	-
Viola palustris	-	II (1-2)	-	-	V (1-4)	-	-	-	-	-	-

Appendix D

Reproductive characteristics

Table D-1 Reproductive characteristics of *Cirsium dissectum* from 22 sites in the British Isles.

	Popn. area m^2	Density m^2 all quadrats (used to calculate total number of rosettes)	Density m^2 quadrats with *C. dissectum*	Total no. of rosettes	No. of flowering rosettes	Prop. of flowering rosettes %	Mean no. of hard seeds per capitulum (SE)	Mean seed mass mg (SE)	Mean germination % (SE)	Mean seedling survival % (SE)	No. of seed heads predated %
EAC	12827	3	8	39335	110	3.3	82.7 (11.5)	2.65 (0.09)	12.6 (10.1)	8.9 (8.9)	0
EBB	1677	12	24	19896	210	0.8	26.1 (4.6)	2.29 (0.19)	2.7 (2.1)	0.0 (0.0)	10
EKS	56346	12	17	676158	4565	2.5	51.6 (6.7)	2.99 (0.11)	3.1 (1.6)	25.6 (15.5)	3
EMM	14265	6	9	90821	4964	6.8	32.9 (6.4)	2.66 (0.27)	3.0 (1.6)	44.8 (27.0)	20
EMO	98935	6	10	619994	63	0.0	22.7 (4.9)	1.32 (0.17)	6.7 (3.7)	0.0 (0.0)	0
EMS	7865	7	13	52959	463	4.5	36.8 (5.2)	2.56 (0.18)	4.9 (2.7)	30.5 (17.5)	17
ERW	54942	10	17	575065	26	0.6	10.5 (1.5)	1.27 (0.00)	0.0 (0.0)		0
EWF	981	3	14	3138	308	14.6	39.6 (5.5)	2.90 (0.27)	6.6 (4.7)	7.6 (5.3)	4
IAR	2518	2	4	6212	350	5.4	70.7 (6.8)	3.63 (0.19)	29.3 (6.5)	55.7 (7.6)	13
IBL	6478	4	5	23537	200	7.3	40.8 (6.8)	2.94 (0.14)	23.8 (8.3)	46.5 (9.4)	0
IDL	10653	8	12	88063	1107	2.4	49.0 (4.4)	3.05 (0.10)	2.4 (1.1)	33.3 (13.1)	3
IGC	63697	19	20	1184757	5500	3.0	20.9 (2.3)	2.90 (0.19)	11.7 (4.5)	34.3 (12.2)	0
ILB	7345	8	8	57292	244	1.3	10.4 (2.9)	3.49 (0.21)	28.8 (8.9)	47.4 (13.9)	63
ILG	13753	6	9	79769	550	1.7	29.0 (3.9)	2.18 (0.14)	13.8 (3.9)	14.9 (6.3)	0
ILL	43697	4	5	163135	21	0.0	55.0 (41.0)	2.48 (0.21)	0.0 (0.0)		0
ILT	1051	4	11	3783	460	4.6	37.4 (4.9)	2.30 (0.20)	0.7 (0.4)	66.7 (23.9)	17
IMA	5647	4	6	21270	19	0.9	18.0 (18.0)	3.07 (0.00)	1.9 (0.0)	0.0 (0.0)	0
IME	1409	8	12	11881	350	3.2	26.5 (6.4)	2.33 (0.19)	13.4 (6.4)	69.2 (8.4)	7
WCG	16624	2	4	38788	1751	2.9	29.9 (4.2)	2.84 (0.12)	8.1 (2.3)	11.7 (5.0)	3
WDB	16419	8	15	125877	2808	7.0	30.8 (5.0)	2.15 (0.08)	10.8 (5.1)	34.4 (13.9)	7
WKF	7711	4	19	31358	4180	23.0	6.5 (1.6)	2.77 (0.28)	1.6 (1.4)	0.0 (0.0)	10
WWM	3196	3	6	7990	519	6.7	11.2 (2.5)	2.81 (0.39)	3.8 (2.3)	11.1 (11.1)	13
Mean	**20365**	**6**	**11**	**178231**	**1308**	**4.7**	**33.6**	**2.62**	**8.6**	**27.1**	**9**
SD	**26107**	**4**	**6**	**305013**	**1816**	**5.3**	**19.4**	**0.57**	**8.8**	**22.6**	**14**

Appendix E

Clone maps

Each 'clone map' is a schematic representation of the genotypes found within a *Cirsium dissectum* population. Thirty-five leaf samples were collected using a systematic sampling strategy and genotyped using 7 microsatellite loci. Black crosses represent plants with unique genotypes. Plants that shared the same multilocus genotype (IMLG) are shown with the same symbol. For each IMLG the P_{gen} value is given; this provides the probability that the same genotype could have arisen through sexual recombination (section 3.2.3). For all IMLGs the P_{gen} values were lower than 0.05, so plants sharing the same multilocus genotypes are considered to be part of the same clone.

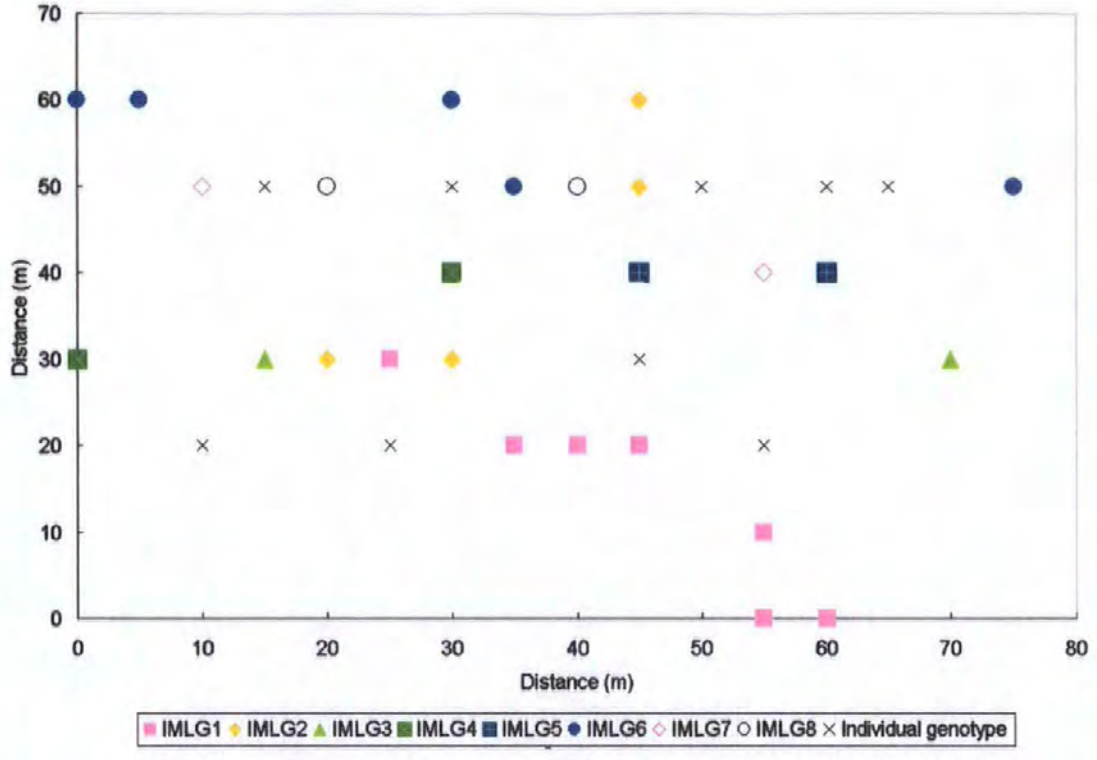


Figure E-1 Braunton Burrows (EBB) P_{gen} for each identical multilocus genotype (IMLG): 1 = 0.0490; 2 = 0.0482; 3 = 0.0461; 4 = 0.0234; 5 = 0.037; 6 = 0.0294; 7 = 0.0481; 8 = 0.0471.

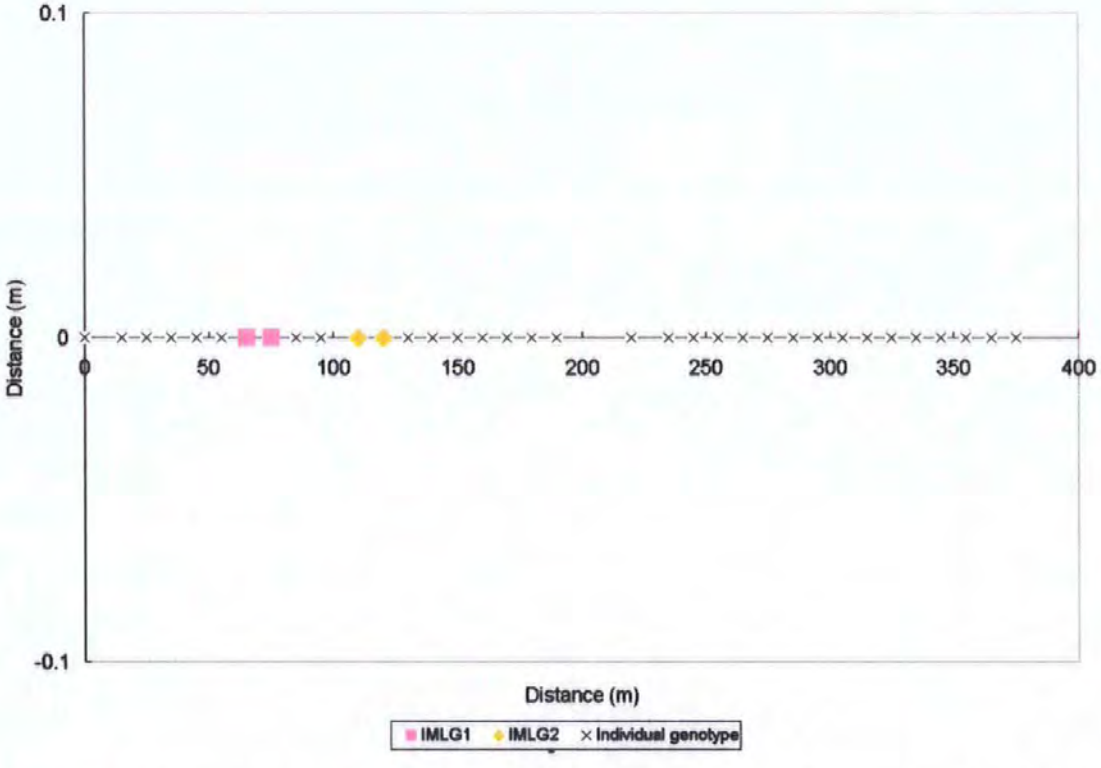


Figure E-2 Knowstone Moor (EKS) P_{gen} for each identical multilocus genotype (IMLG): 1 = 0.0012; 2 = 0.0001.

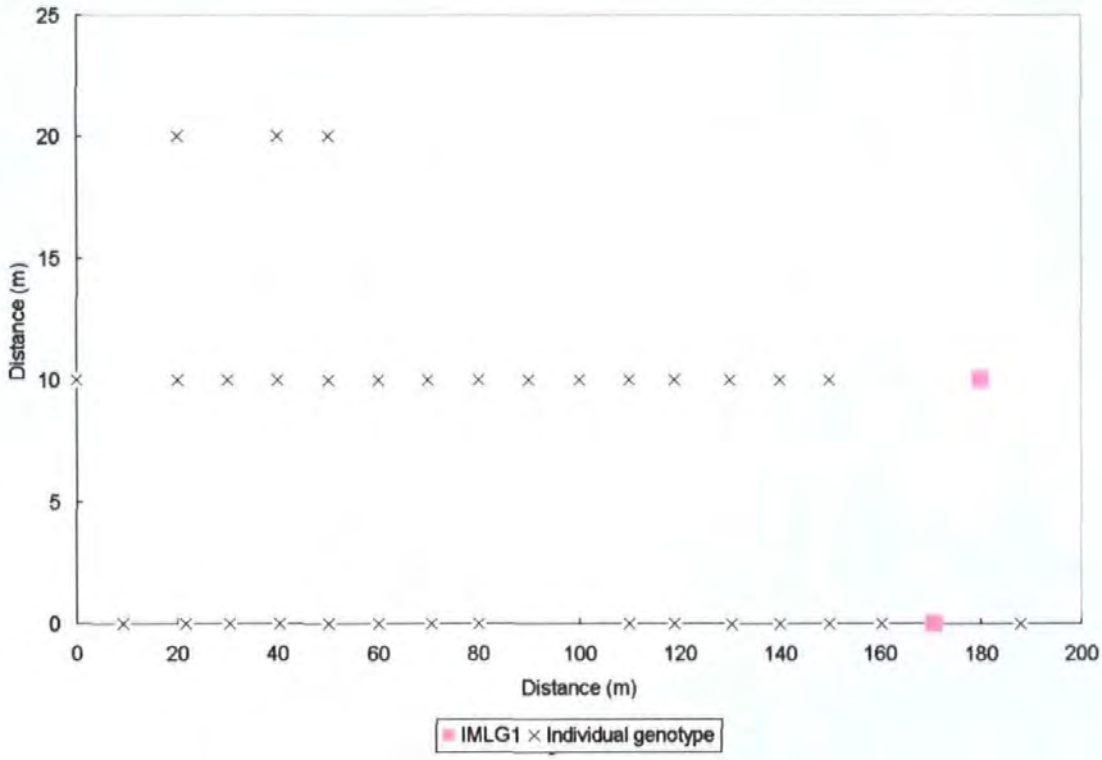


Figure E-3 Mambury Moor (EMM) P_{gen} for each identical multilocus genotype (IMLG): 1 = 0.0001

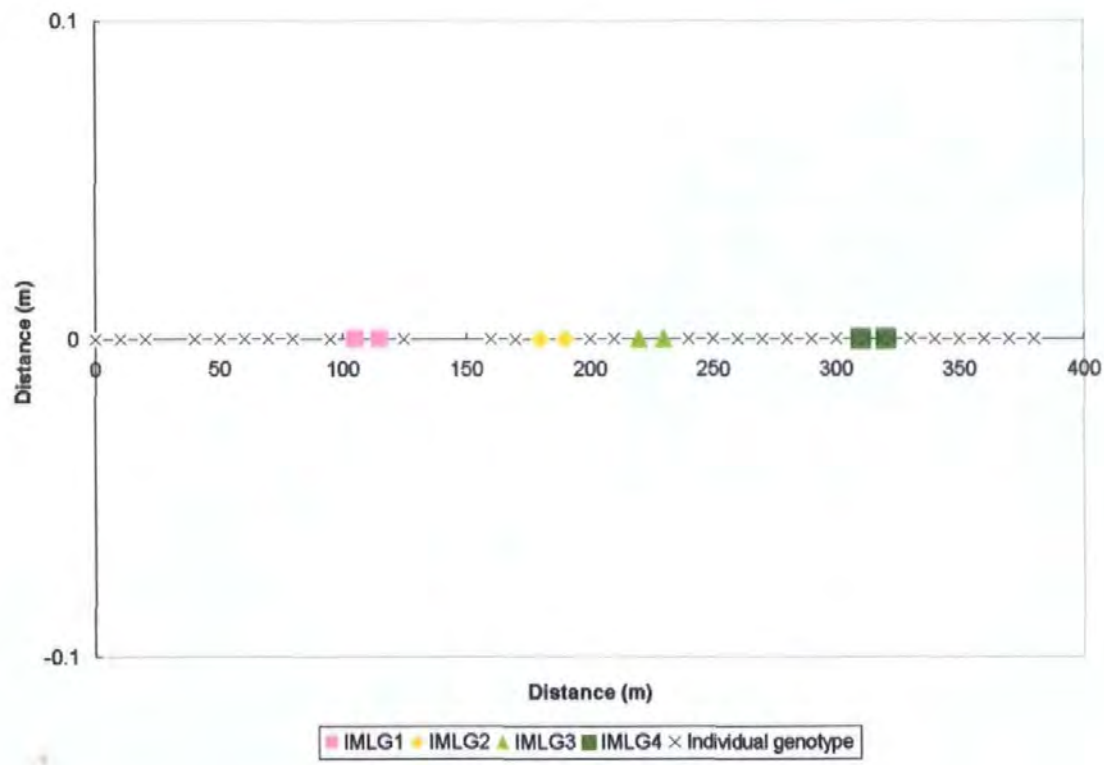


Figure E-4 Marlpitt Oak (EMO) P_{gen} for each identical multilocus genotype (IMLG): 1 = 0.0004; 2 = 4.21 x10 $^{-6}$; 3 = 9.53 x10 $^{-5}$; 4 = 0.0002.

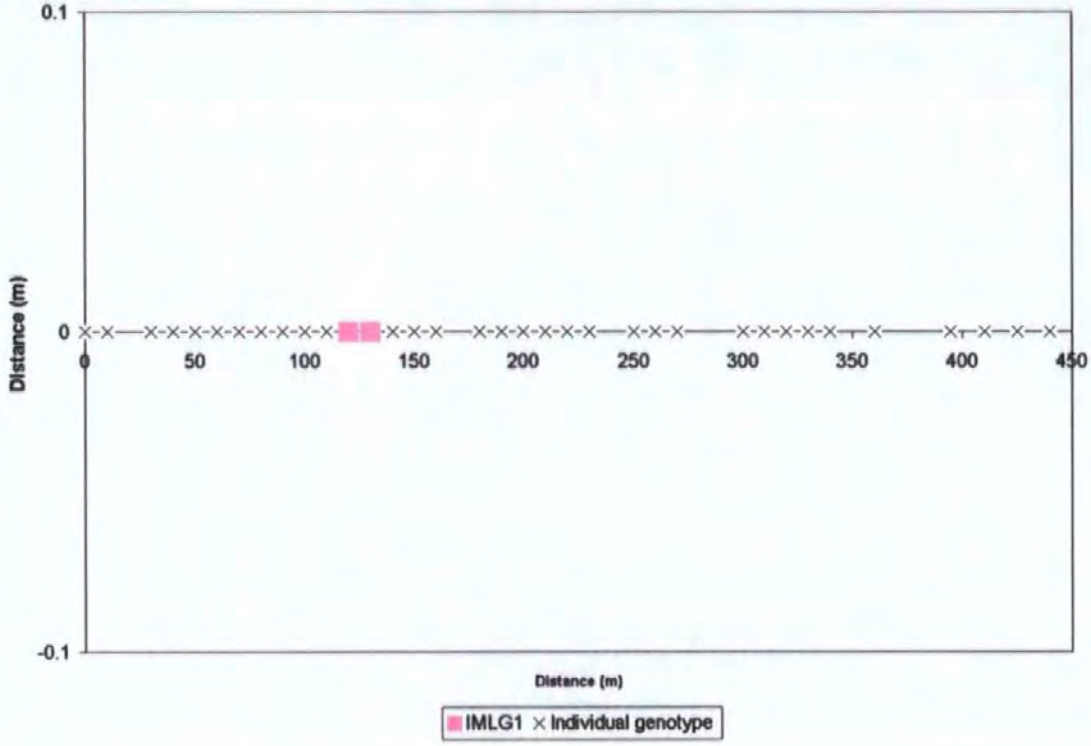


Figure E-5 Rans Wood (ERW) P_{gen} for each identical multilocus genotype (IMLG): 1 = 3.02×10^{-6}.

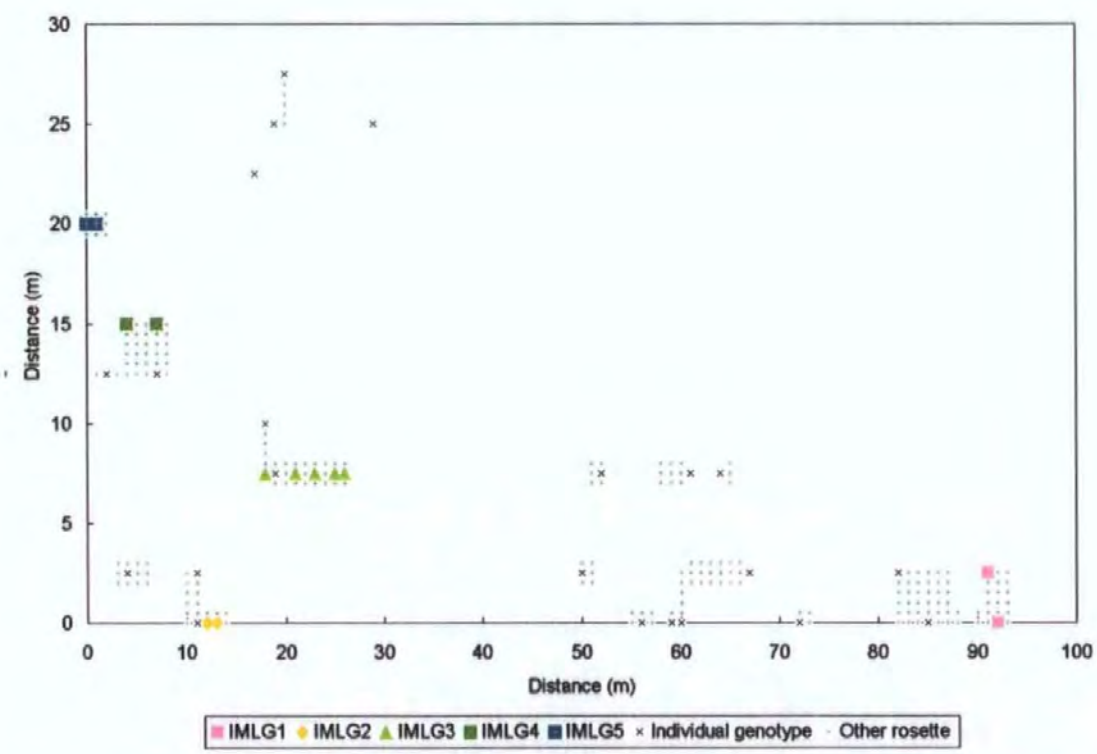


Figure E-6 Wicken Fen (EWF) P_{gen} for each identical multilocus genotype (IMLG): 1 = 0.0011; 2 = 0.0021; 3 = 0.0232; 4 = 0.0113; 5 = 0.0051.

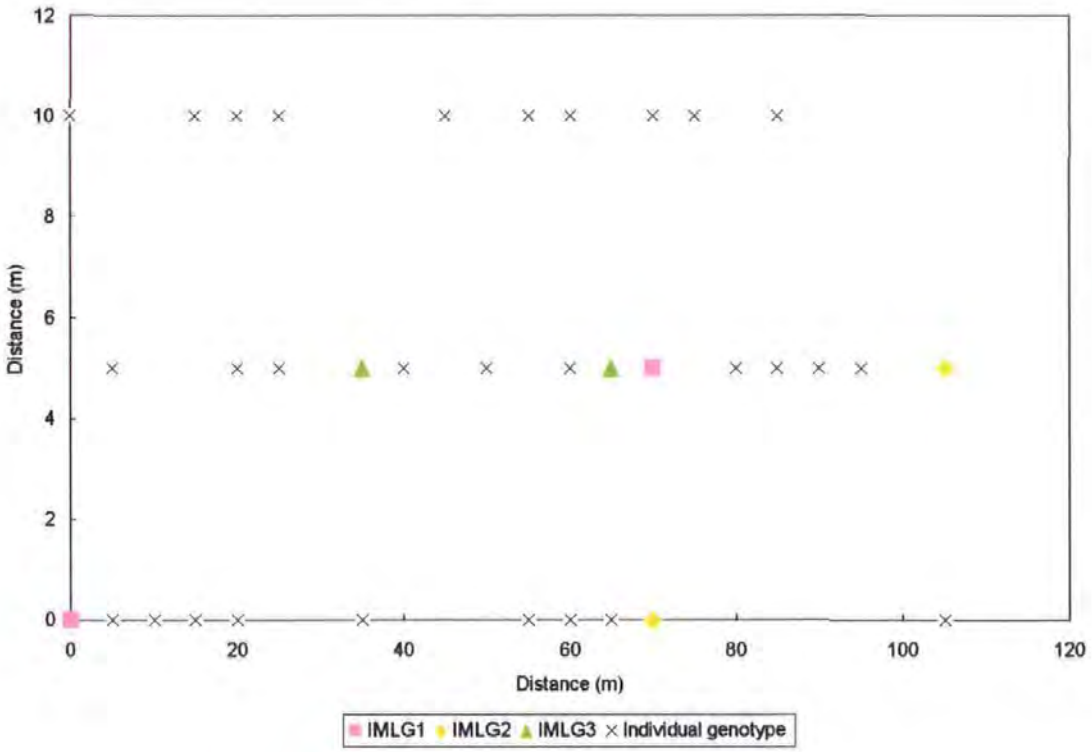


Figure E-7 Lough Corrib (IAR) P_{gen} for each identical multilocus genotype (IMLG): 1 = 7.75 x10 $^{-5}$; 2 = 0.0041; 3 = 0.0201.

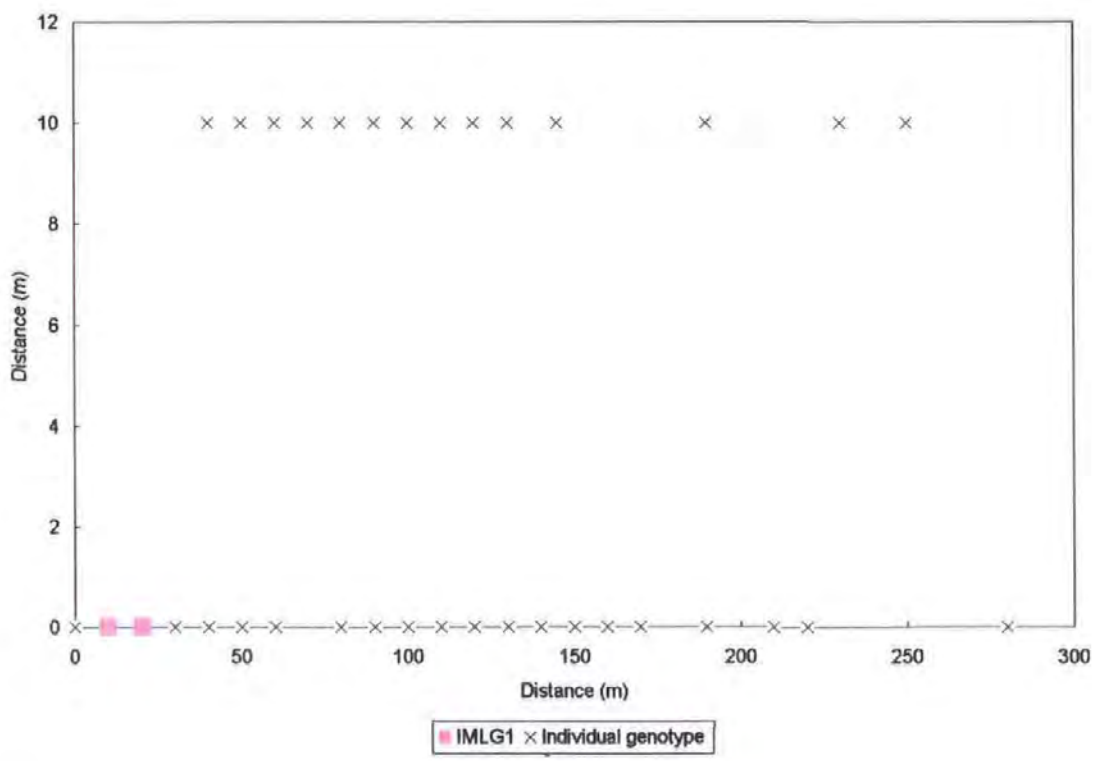


Figure E-8 Dough Lough (IDL) P_{gen} for each identical multilocus genotype (IMLG): 1 = 1.19 x 10^{-5}.

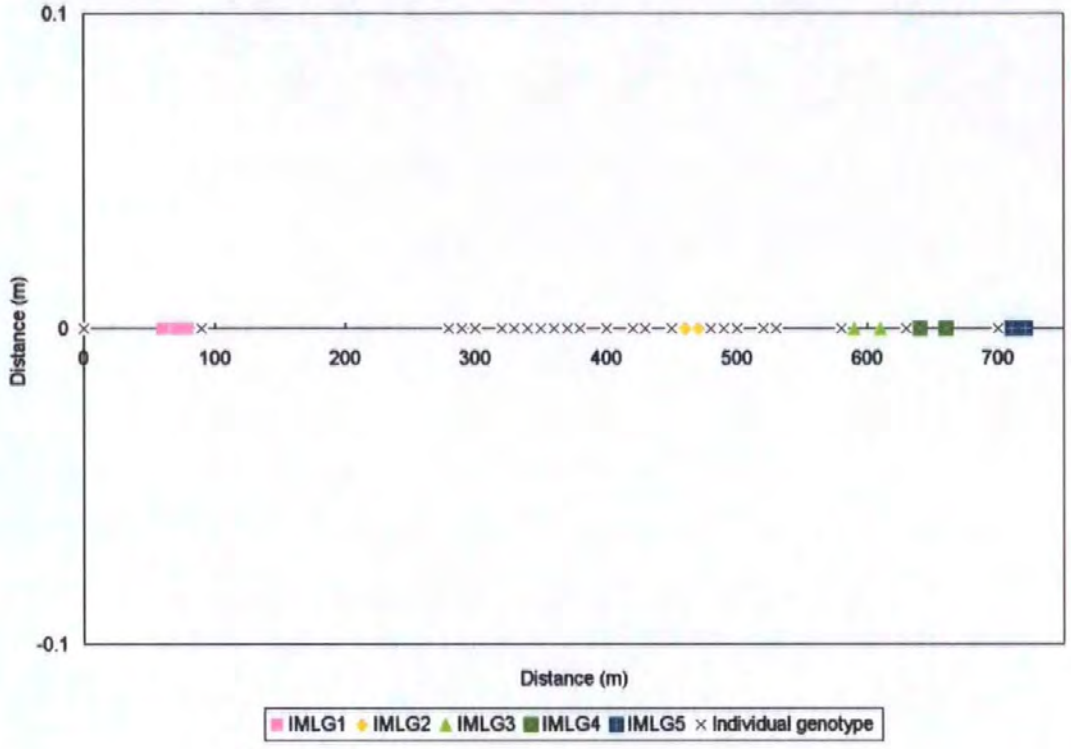


Figure E-9 Giant's Causeway (IGC) P_{gen} for each identical multilocus genotype (IMLG): 1 = 0.0007; 2 = 0.0054; 3 = 0.0075; 4 = 0.0022; 5 = 0.0007.

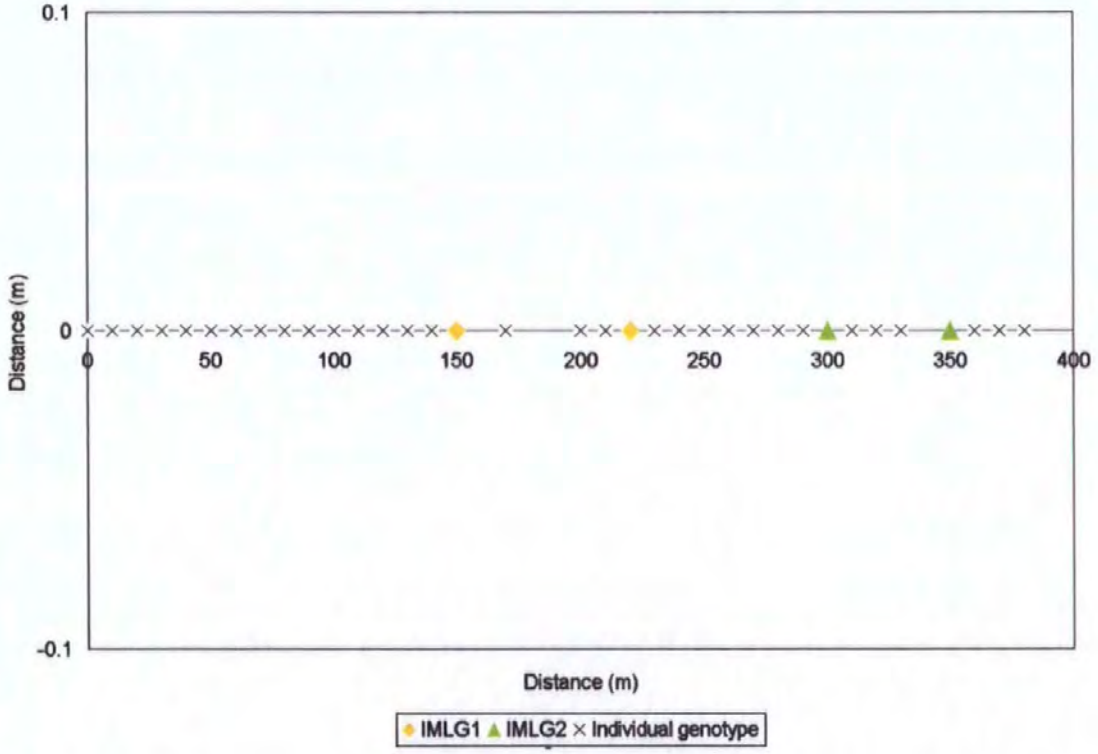


Figure E-10 Lough Gealain (ILG) P_{gen} for each identical multilocus genotype (IMLG): 1 = 0.0085; 2 = 0.0029.

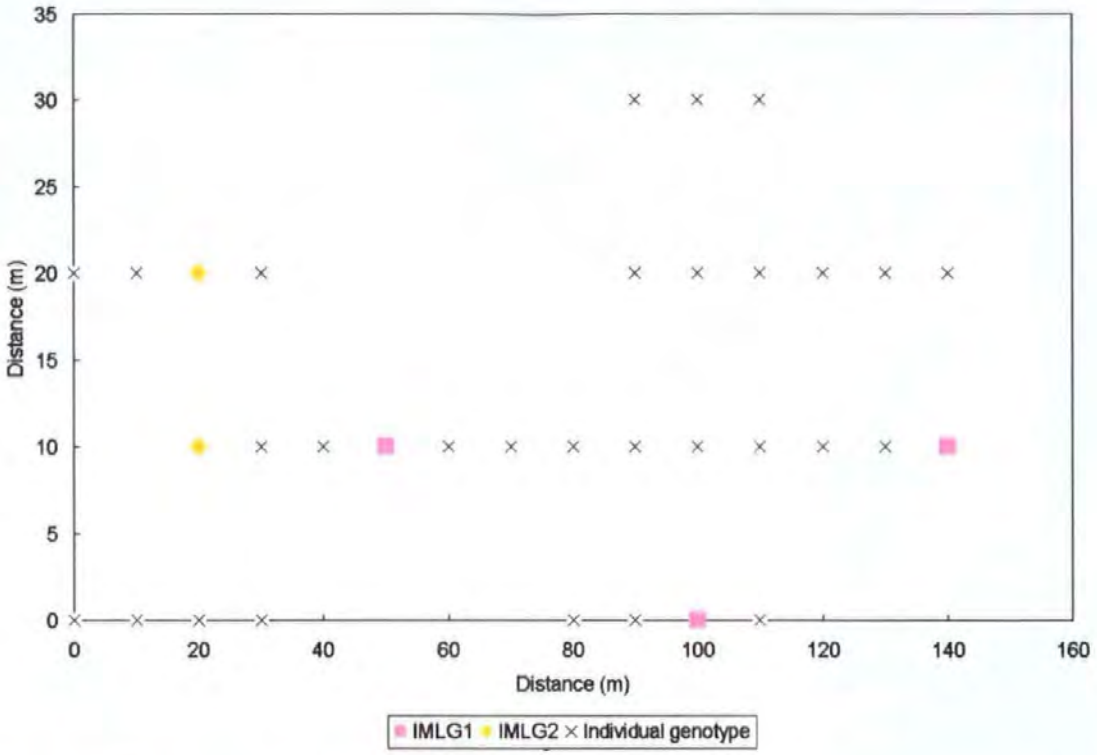


Figure E-11 Marble Arch (IMA) P_{gen} for each identical multilocus genotype (IMLG): 1 = 0.0023; 2 = 0.0002.

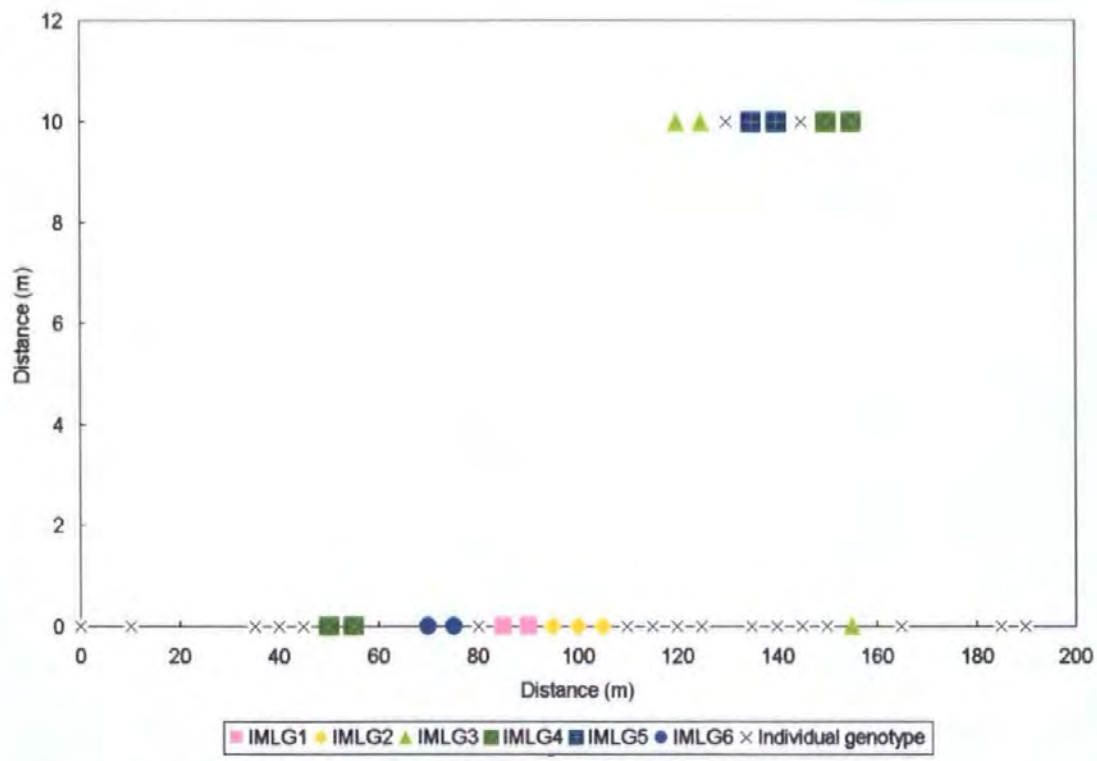


Figure E-12 Meencargagh (IME) P_{gen} for each identical multilocus genotype (IMLG): 1 = 0.0003; 2 = 0.0027; 3 = 0.0048; 4 = 0.0036; 5 = 8.40 x10^{-5}; 6 = 0.0018

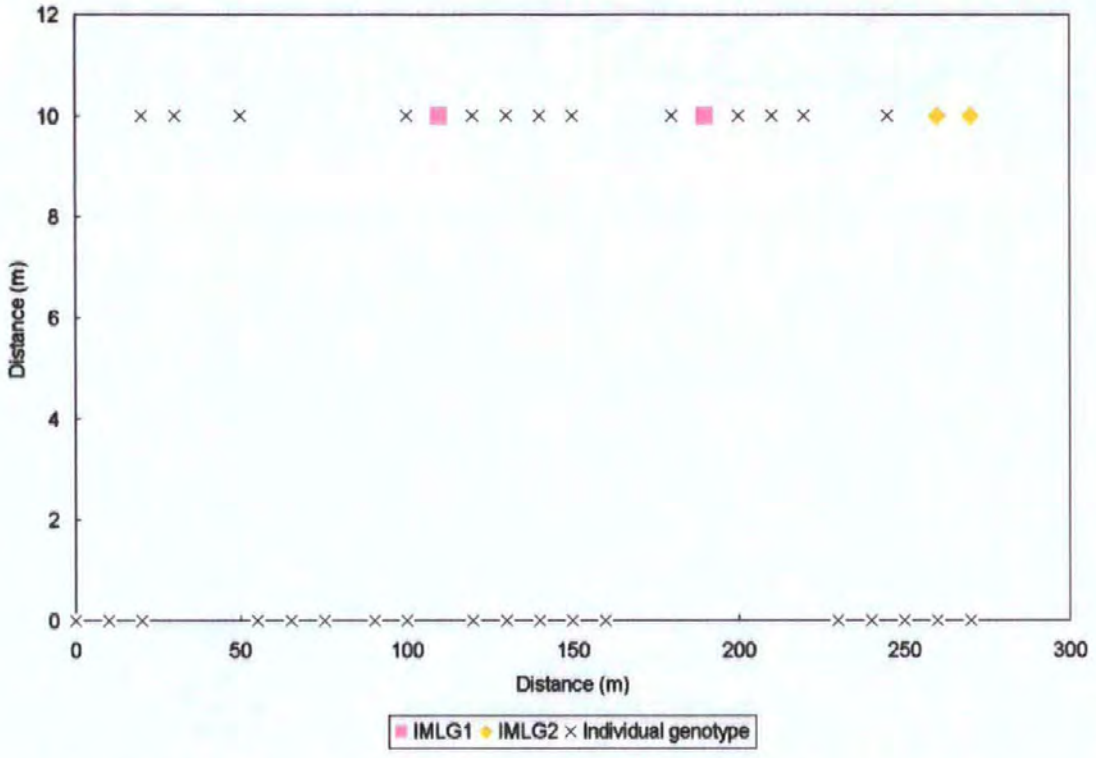


Figure E-13 Drostre Bank (WDB) P_{gen} for each identical multilocus genotype (IMLG): 1 = 0.0086; 2 = 0.0090.

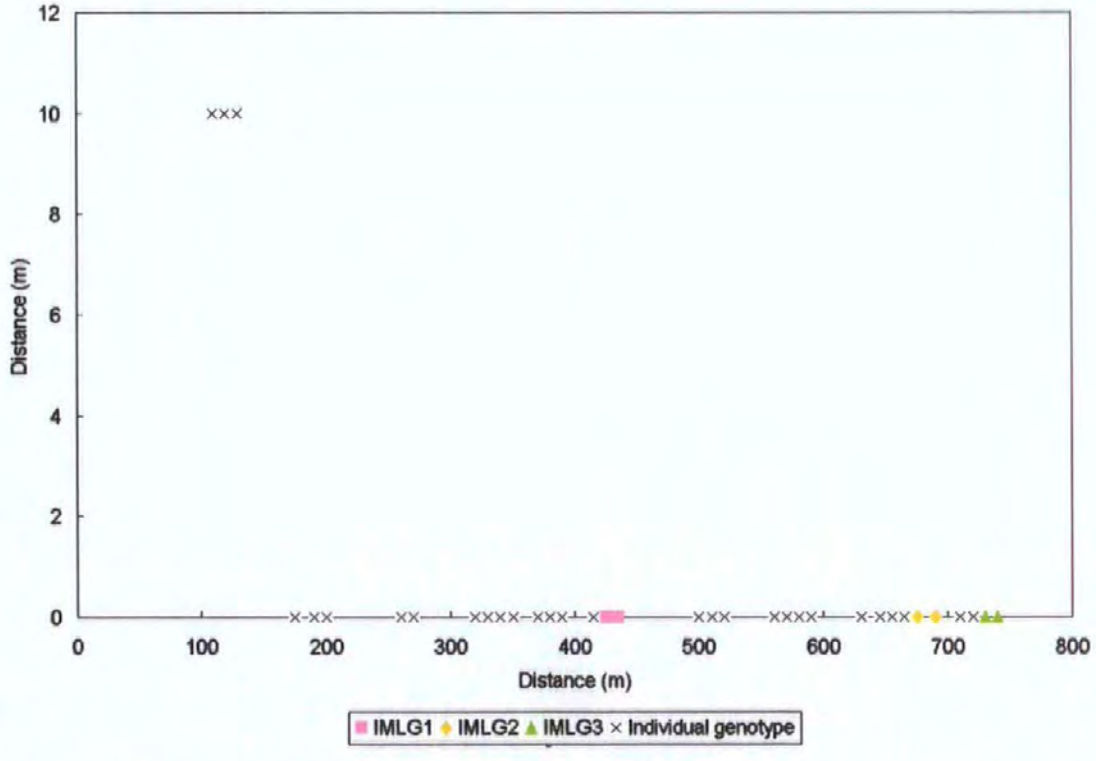


Figure E-14 Kenfig (WKF) P_{gen} for each identical multilocus genotype (IMLG): 1 = 0.0004; 2 = 0.0017; 3 = 0.0013.

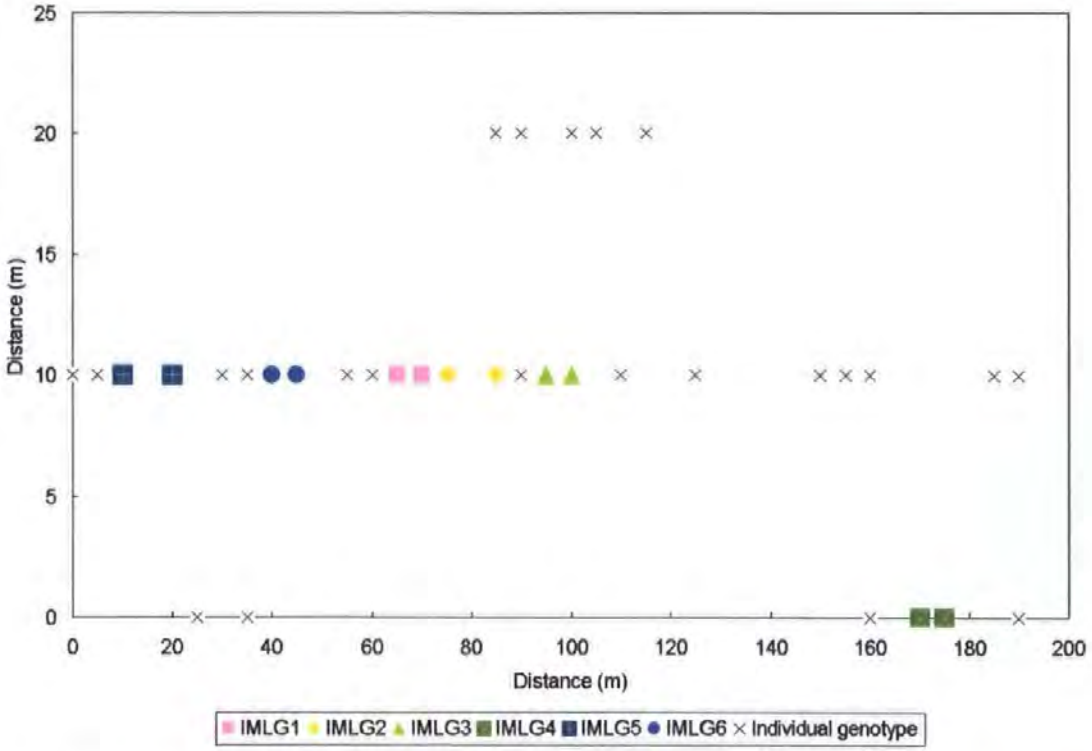


Figure E-15 Welsh Moor (WMM) P_{gen} for each identical multilocus genotype (IMLG): 1 = 0.0036; 2 = 0.0015; 3 = 0.0122; 4 = 0.0206; 5 = 0.0213; 6 = 0.0011.

Appendix F

Genetic and geographic distances

Table F-1 Nei's (1972) genetic distance (top) vs geographical distance in Km (bottom) – for genets

	EAC	EBB	EKS	EMA	EMO	EMS	ERW	EWF	IAR	IBL	IDL	IGC	ILB	ILG	ILL	ILT	IMA	IME	WCG	WDB	WKF	WWM
EAC		0.205	0.082	0.228	0.117	0.076	0.134	0.444	0.537	0.610	0.439	0.760	0.458	0.563	0.523	0.620	0.598	0.629	0.097	0.235	0.067	0.130
EBB	73.926		0.376	0.304	0.331	0.415	0.317	0.771	1.525	1.516	0.910	1.473	1.093	1.462	1.164	1.394	1.040	1.391	0.387	0.702	0.351	0.412
EKS	36.971	40.802		0.263	0.156	0.064	0.200	0.269	0.415	0.478	0.497	0.657	0.402	0.403	0.502	0.522	0.677	0.541	0.161	0.168	0.118	0.179
EMA	69.353	23.654	46.033		0.213	0.295	0.174	0.232	0.573	0.615	0.440	0.742	0.417	0.652	0.577	0.575	0.617	0.615	0.237	0.389	0.338	0.334
EMO	123.456	185.681	145.476	189.438		0.073	0.082	0.313	0.339	0.464	0.335	0.664	0.297	0.408	0.363	0.502	0.535	0.522	0.152	0.228	0.107	0.154
EMS	40.374	34.157	8.968	37.145	153.544		0.152	0.321	0.315	0.423	0.370	0.694	0.304	0.360	0.373	0.496	0.608	0.520	0.137	0.139	0.070	0.136
ERW	131.646	193.009	152.988	197.234	8.493	161.152		0.268	0.341	0.430	0.273	0.584	0.261	0.408	0.348	0.410	0.423	0.451	0.109	0.181	0.092	0.096
EWF	307.614	337.446	308.925	353.114	212.112	317.746	205.048		0.334	0.446	0.555	0.737	0.283	0.359	0.623	0.448	0.809	0.561	0.296	0.243	0.358	0.367
IAR	504.900	431.131	469.393	441.416	599.253	464.597	604.511	652.295		0.110	0.145	0.224	0.036	0.027	0.156	0.102	0.278	0.130	0.364	0.173	0.310	0.328
IBL	435.526	361.806	401.737	368.883	539.127	395.912	545.158	618.504	91.769		0.113	0.117	0.124	0.120	0.068	0.044	0.181	0.065	0.477	0.291	0.407	0.432
IDL	514.322	445.299	477.352	461.739	585.078	475.026	588.611	590.683	142.046	207.012		0.162	0.097	0.155	0.049	0.107	0.095	0.085	0.298	0.254	0.288	0.288
IGC	547.589	487.107	511.756	507.290	593.881	511.640	595.673	553.232	269.643	327.125	127.716		0.224	0.177	0.139	0.080	0.136	0.024	0.649	0.528	0.591	0.641
ILB	461.901	387.977	427.037	396.986	560.468	421.797	566.085	626.198	50.168	42.625	169.662	293.416		0.049	0.161	0.092	0.229	0.118	0.272	0.169	0.264	0.302
ILG	466.114	392.190	431.408	400.876	565.455	426.073	571.129	632.572	50.214	41.556	174.776	299.138	7.068		0.170	0.086	0.232	0.101	0.389	0.195	0.324	0.375
ILL	514.364	444.852	477.395	460.950	586.771	474.905	590.421	595.415	133.195	199.513	9.046	136.677	161.655	166.649		0.112	0.116	0.080	0.422	0.307	0.355	0.356
ILT	533.545	461.268	496.940	474.690	616.835	493.309	621.244	644.638	76.166	160.451	77.246	202.425	118.182	121.014	68.372		0.102	0.035	0.432	0.301	0.401	0.452
IMA	498.354	428.910	461.384	445.085	570.883	458.912	574.562	581.340	131.753	191.738	17.895	138.690	155.615	161.050	16.031	74.302		0.112	0.445	0.461	0.435	0.462
IME	522.425	455.299	485.549	472.866	587.040	483.806	590.132	580.725	175.177	238.212	33.133	94.694	201.863	207.166	42.019	108.573	46.479		0.481	0.363	0.441	0.495
WCG	94.484	63.191	62.095	85.396	164.455	65.736	169.969	282.260	434.869	375.786	428.505	454.532	396.118	401.176	429.324	454.796	413.304	434.099		0.112	0.062	0.069
WDB	141.076	115.820	113.266	138.623	177.382	118.142	180.716	248.607	431.602	382.299	407.909	420.381	397.285	402.958	409.789	442.557	393.960	409.689	53.565		0.100	0.084
WKF	94.651	56.739	60.503	79.530	170.663	63.057	176.384	290.617	429.001	368.966	424.914	453.215	389.806	394.795	425.574	450.001	409.546	431.094	8.390	59.122		0.050
WWM	115.402	58.337	78.792	81.696	199.589	78.320	205.375	313.090	400.436	339.938	399.790	433.323	360.923	365.876	400.137	422.743	384.107	407.181	36.147	69.607	29.029	

Table F-2 Nei's (1972) genetic distance (top) vs geographical distance in Km (bottom) – for ramets

	EAC	EBB	EKS	EMA	EMO	EMS	ERW	EWF	IAR	IBL	IDL	IGC	ILB	ILG	ILL	ILT	IMA	IME	WCG	WDB	WKF	WWM
EAC		0.231	0.086	0.228	0.109	0.076	0.134	0.448	0.544	0.610	0.440	0.747	0.458	0.564	0.523	0.620	0.606	0.646	0.097	0.239	0.063	0.133
EBB	73.926		0.410	0.326	0.348	0.447	0.356	0.801	1.618	1.563	0.951	1.523	1.136	1.537	1.199	1.426	1.085	1.473	0.436	0.744	0.378	0.483
EKS	36.971	40.802		0.263	0.151	0.061	0.199	0.259	0.419	0.484	0.504	0.642	0.402	0.403	0.509	0.532	0.694	0.563	0.163	0.172	0.117	0.174
EMA	69.353	23.654	46.033		0.223	0.298	0.175	0.236	0.591	0.616	0.444	0.728	0.422	0.660	0.574	0.577	0.617	0.610	0.238	0.387	0.344	0.339
EMO	123.456	185.681	145.476	189.438		0.073	0.081	0.336	0.361	0.483	0.339	0.676	0.307	0.420	0.377	0.518	0.539	0.560	0.145	0.236	0.096	0.148
EMS	40.374	34.157	8.968	37.145	153.544		0.149	0.333	0.321	0.423	0.366	0.669	0.304	0.356	0.373	0.496	0.605	0.540	0.137	0.143	0.065	0.127
ERW	131.646	193.009	152.988	197.234	8.493	161.152		0.272	0.341	0.430	0.272	0.578	0.255	0.401	0.351	0.409	0.426	0.467	0.106	0.180	0.093	0.099
EWF	307.614	337.446	308.925	353.114	212.112	317.746	205.048		0.378	0.493	0.578	0.721	0.306	0.384	0.665	0.484	0.826	0.573	0.290	0.247	0.381	0.365
IAR	504.900	431.131	469.393	441.416	599.253	464.597	604.511	652.295		0.113	0.141	0.202	0.039	0.023	0.158	0.103	0.266	0.124	0.371	0.177	0.322	0.310
IBL	435.526	361.806	401.737	368.883	539.127	395.912	545.158	618.504	91.769		0.109	0.108	0.124	0.116	0.068	0.044	0.171	0.089	0.477	0.288	0.415	0.408
IDL	514.322	445.299	477.352	461.739	585.078	475.026	588.611	590.683	142.046	207.012		0.158	0.094	0.148	0.045	0.101	0.094	0.100	0.297	0.246	0.290	0.279
IGC	547.589	487.107	511.756	507.290	593.881	511.640	595.673	553.232	269.643	327.125	127.716		0.205	0.160	0.141	0.070	0.140	0.024	0.627	0.490	0.585	0.607
ILB	461.901	387.977	427.037	396.986	560.468	421.797	566.085	626.198	50.168	42.625	169.662	293.416		0.048	0.161	0.092	0.225	0.112	0.272	0.166	0.273	0.287
ILG	466.114	392.190	431.408	400.876	565.455	426.073	571.129	632.572	50.214	41.556	174.776	299.138	7.068		0.170	0.086	0.231	0.092	0.391	0.191	0.333	0.349
ILL	514.364	444.852	477.395	460.950	586.771	474.905	590.421	595.415	133.195	199.513	9.046	136.677	161.655	166.649		0.112	0.114	0.111	0.422	0.307	0.359	0.341
ILT	533.545	461.268	496.940	474.690	616.835	493.309	621.244	644.638	76.166	160.451	77.246	202.425	118.182	121.014	68.372		0.093	0.035	0.432	0.297	0.414	0.433
IMA	498.354	428.910	461.384	445.085	570.883	458.912	574.562	581.340	131.753	191.738	17.895	138.690	155.615	161.050	16.031	74.302		0.115	0.450	0.451	0.448	0.457
IME	522.425	455.299	485.549	472.866	587.040	483.806	590.132	580.725	175.177	238.212	33.133	94.694	201.863	207.166	42.019	108.573	46.479		0.483	0.365	0.467	0.498
WCG	94.484	63.191	62.095	85.396	164.455	65.736	169.969	282.260	434.869	375.786	428.505	454.532	396.118	401.176	429.324	454.796	413.304	434.099		0.113	0.064	0.076
WDB	141.076	115.820	113.266	138.623	177.382	118.142	180.716	248.607	431.602	382.299	407.909	420.381	397.285	402.958	409.789	442.557	393.960	409.689	53.565		0.103	0.077
WKF	94.651	56.739	60.503	79.530	170.663	63.057	176.384	290.617	429.001	368.966	424.914	453.215	389.806	394.795	425.574	450.001	409.546	431.094	8.390	59.122		0.049
WWM	115.402	58.337	78.792	81.696	199.589	78.320	205.375	313.090	400.436	339.938	399.790	433.323	360.923	365.876	400.137	422.743	384.107	407.181	36.147	69.607	29.029	

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Biological Flora of the British Isles: *Cirsium dissectum* (L.) Hill (*Cirsium tuberosum* (L.) All. subsp. *anglicum* (Lam.) Bonnier; *Cnicus pratensis* (Huds.) Willd., non Lam.; *Cirsium anglicum* (Lam.) DC.)

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Summary

1 This account reviews information on all aspects of the biology of *Cirsium dissectum* (L.) Hill that are relevant to understanding its ecological characteristics and behaviour. The main topics are presented within the standard framework of the *Biological Flora of the British Isles*: distribution, habitat, communities, responses to biotic factors, responses to environment, structure and physiology, phenology, floral and seed characters, herbivores and disease, history and conservation.

2 *Cirsium dissectum* (meadow thistle) is a perennial, rhizomatous herb found in moist, nutrient poor grasslands and heathlands in north-west Europe. It is readily distinguishable from other *Cirsium* species in the British Isles but has been considered a subspecies of *C. tuberosum*, along with *C. filipendulum*, in some other areas of Europe.

3 It is susceptible to being out-competed by species that are able to increase biomass more rapidly. At more productive sites, greater nutrient availability increases the proportion of rosettes that flower, as well as rosette turnover. Seeds germinate readily under a range of conditions in the growth room and greenhouse but seedlings are very rarely found in the field. An examination of its population dynamics reveals that clonal propagation is the dominant form of reproduction, with the low number of seedlings primarily caused by very low establishment rates in vegetation stands.

4 *Cirsium dissectum* is relatively tolerant of drought and shade even though it is found in moist grasslands. At very low pH it suffers from ammonium and aluminium toxicity. As it has suffered habitat loss through drainage and succession, *C. dissectum* has declined in the British Isles and it is now endangered in Germany and the Netherlands.

Key-words: *Cirsium dissectum*, climatic limitation, communities, conservation, ecophysiology, geographical and altitudinal distribution, germination, herbivory, mycorrhiza, parasites and diseases, reproductive biology, soils

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*Abbreviated references are used for many standard works: see *Journal of Ecology* (1975), **63**, 335–344. Nomenclature of vascular plants follows *Flora Europaea* and, where different, Stace (1997).

Sect. *Cirsium* (Sect. *Chamaeleon* DC.). Meadow thistle. A perennial herb with short obliquely ascending stock, cylindrical roots and rhizomes up to 40 cm long. The basal-rosette leaves are 8–25 × 2–3 cm, elliptical-lanceolate, long stalked, sinuate-toothed or slightly pinnatifid. Leaves are slightly hairy above and whitish

cottony beneath with margins bearing soft prickles that are longest on the teeth or lobes. Leaves are not decurrent. The flowering stem is erect, usually simple, terete, cottony and unwinged, 6–80 cm tall. There are usually a few small bract-like leaves above the middle; these are like the basal leaves but oblong–lanceolate and semi-amplexicaul with basal auricles. Capitula are 2.5–3 × 2–2.5 cm, usually solitary. The involucre is ovoid, purplish and cottony with bracts that are lanceolate and appressed, the outer bracts being spine-tipped, the inner acuminate. Flowers are magenta-purple and hermaphrodite. Achenes 3–4 mm long, 1.3–3.6 mg, pale fawn, smooth with a long, pure-white pappus.

Fl. Eur. **4** places *C. dissectum* within the *C. tuberosum* (L.) All. group, along with *C. tuberosum* and *C. filipendulum* Lange. Within the British Isles *C. filipendulum* is not found, and *C. dissectum* is readily distinguishable from *C. tuberosum* by its less pinnatifid leaves, long rhizomes and absence of tuberous roots. All three species occur in Germany, but not in the same sites (Hegi Fl. ed. 6, **2**). In France the three species can be difficult to distinguish and Fl Eur. **4** considers that they could probably be treated as subspecies. Rouy (1905) recognized four forms of *C. anglicum* (synonym of *C. dissectum*): f. *typicum* with regular teethed leaves; f. *angustifolium* with almost linear leaves; f. *dissectum* with irregularly incised or lobed leaves, being more robust than the previous two forms; and f. *ambiguum* with very pinnatifid leaves and strong growth, often with 2–3 stems. Hegi Fl. ed. 6, **2** also describes high levels of variation in the dissection of the leaves. Sell & Murrell (2006) recognize no variants; plants from the British Isles, when grown in standard common garden conditions, typically show only slightly pinnatifid leaves but some populations have recognizably more pinnatifid leaf forms (de Vere 2007).

Cirsium dissectum is largely confined to oligotrophic, weakly to strongly calcareous, wet grasslands and heathlands, fens and dune slacks, often on peaty soils. It tends to grow on sites subject to flushes of base-rich water (Preston *et al.* 2002).

I. Geographical and altitudinal distribution

Cirsium dissectum is an Oceanic West European (Dist. Br. Fl.) or Oceanic Temperate (Preston & Hill 1997) plant, concentrated in the British Isles in south-west England, south Wales and western Ireland (Fig. 1). It is found frequently in Devon, Dorset and Hampshire (particularly the New Forest) and in South Wales. It becomes patchy further north and east, has declined greatly since 1930 (Preston *et al.* 2002) and is still in decline since 1970 (Fig. 1). It is apparently extinct in 8 vice-counties in eastern England (Stace *et al.* 2003) and has been lost from many localities elsewhere, although counties such as Oxfordshire and Berkshire still have a number of sites. The fens of Norfolk and to a lesser extent Suffolk still represent a stronghold for the species. It becomes sporadic as it extends northwards but it has a number of sites in Yorkshire, north to Roxby, 54°32′ N. In Scotland it is found only on Islay and south-east Jura and the adjacent mainland of Kintyre, where it may have colonized naturally from Northern Ireland or possibly been introduced. *Cirsium dissectum* is frequent throughout western Ireland, but it is rare and declining in the north-east (Hackney 1992). Its European distribution is western (Fig. 2): it is found in the Netherlands (Rossenaar & Groen 2003) and has a limited distribution in Belgium (Van Rompaey & Delvosalle 1972) and north-west Germany (Haeupler & Schonfelder 1989). It extends southwards through France, being relatively common (at least formerly) throughout the west, north and centre of the country (Bonnier 1851–1922). In Germany 57% of populations have become extinct since 1930 (Buck-Sorlin 1993) and strong declines have also occurred in the Netherlands (Rossenaar & Groen 2003) and in eastern sites in northern France (Institut floristique Franco-Belge 1995).

Fl. Eur. **4** and Hegi Fl. ed. 6, **4** list *C. dissectum* from Spain but such records are regarded as almost certainly errors by de Bolòs & Vigo (1995). Fl. Eur. **4** describes its occurrence in Italy as doubtful and reports of its presence have not been confirmed (Fiori 1969; Pignatti 1982). It is naturalized in Hungary and Norway (Fl. Eur. **4**) (Fig. 2).

II. Habitat

(A) CLIMATIC AND TOPOGRAPHICAL LIMITATIONS

Cirsium dissectum has an Atlantic distribution, with Ellenberg (1988) classifying it as having a continentality value of 1, which indicates an extremely oceanic species. In Britain, however, it is also found in the warmer south-east, indicating that it has an Oceanic rather than Hyperoceanic distribution (Preston & Hill 1999), distinguishing it from such Atlantic species as *Dryopteris aemula*, *Pinguicula lusitanica* and *Ulex gallii*. Buck-Sorlin (1993) states that the distribution in north-west Germany is determined by three climatic factors: a mean temperature in January greater than 0 °C, an annual fluctuation of mean temperature less than 16 °C and annual precipitation between 600 mm and 800 mm.

Cirsium dissectum is found in sites that are permanently damp and can be found in sites with standing water during the winter months. This corresponds with an Ellenberg water value (recalibrated for British plants) of 8, intermediate between a damp and a wet site indicator (Hill *et al.* 1999).

A lowland species, it has been recorded up to 500 m in County Sligo (Preston *et al.* 2002).

(B) SUBSTRATUM

In the British Isles *C. dissectum* is a characteristic species of rhos pastures. These are wet grasslands occurring on acidic to neutral soils that comprise a

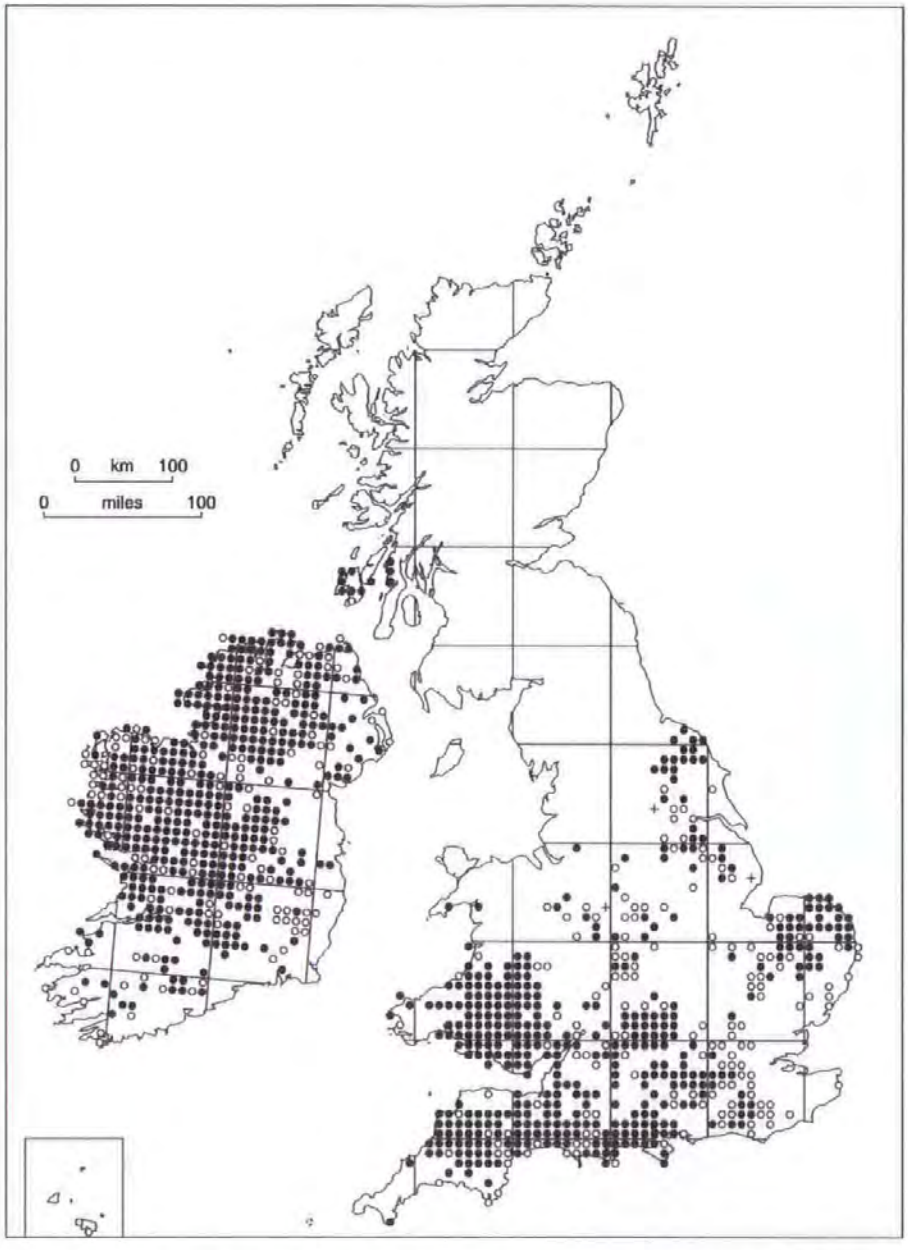

Fig. 1 The distribution of *Cirsium dissectum* in the British Isles. Each dot represents at least one record in a 10 km square of the National Grid. Native: (●) 1970 onwards, (○) pre 1970, (+) introduced. Mapped by H.R. Arnold, using Dr A. Morton's DMAP software, Biological Records Centre, Centre for Ecology & Hydrology, Monks Wood, mainly from data collected by members of the Botanical Society of the British Isles.

mixture of fen meadow, rush pasture and wet heath, often occurring in a mosaic. In Wales these habitats are situated over a wide range of strata ranging from sedimentary and igneous Lower Palaeozoic rocks to Old Red Sandstone, Coal Measures and Carboniferous Limestone of Upper Palaezoic age (Blackstock *et al.* 1998). In Devon and Cornwall rhos pastures are characteristically found on the Culm Measures, sandstones and shales of late Carboniferous age (Durrance & Lamming 1982) that cover much of mid-Devon through to north-east Cornwall. Soil types include poorly draining surface-water gleys with non-humose, humose or peaty topsoils and peats (Blackstock *et al.* 1998; Ross 1999). *Cirsium dissectum* also occurs on sand dune slacks on typical sand-pararendzinas (Ross 1999). In Ireland it extends onto the limestone plateau of the Burren, occurring in the east Burren fens where drainage is impeded (D'Arcy & Hayward 1997).

Mineral nutrient status is typically characterized by particularly low phosphorus and non-limiting concentrations of potassium. Calcium is often abundant in *C. dissectum* sites where it may result from calcareous spring-fed ground water (Wheeler & Shaw 1987). Total nitrogen and organic matter vary considerably from the low concentrations found in sand dunes to high levels in peaty sites (Table 1; de Vere 2007).

Fig. 2 European range of *Cirsium dissectum* (black shading) based on records in the literature (Fl. Eur. **4**; Hegi Fl. ed. 6, **4**; Vergl. Chor.; Bonnier (1851–1922); Haeupler & Schonfelder (1989); Van Rompaey & Delvosalle (1972)). Squares represent countries where it is naturalized.

III. Communities

In Europe, *Cirsium dissectum* is a defining species in the Cirsio-Molinietum Siss. et De Vries 1942. This association has been recorded in Britain (Wheeler 1980) and in Ireland (White & Doyle 1982). These are grasslands dominated by *Molinia caerulea* with *Carex panicea*, *Carex hostiana*, *Carex pulicaris*, *Gymnadenia conopsea*, *Potentilla erecta* and *Succisa pratensis*. In Ireland *C. dissectum* has a wide synecology (O'Criodain & Doyle 1997). White & Doyle (1982) describe it as a characteristic species of the Junco Conglomerati-Molinion Westhoff 1968 and state that most of the wet grasslands in western Ireland belong to this alliance.

Braun-Blanquet & Tüxen (Ir. Pfl.) defined a Cirsio dissecti-Schoenetum nigricantis association that was

Table 1 Soil nutrient characteristics for 22 *Cirsium dissectum* sites throughout the British Isles, sampled in July 2004. Mean with standard deviation in brackets is given along with minimum and maximum values with site name and grid reference

Nutrient	Mean (SD) $n = 22$	Minimum (site, National Grid reference)	Maximum (site, National Grid reference)
Total N (%)	0.7 (0.6)	0.1 (Kenfig, Wales, SS784816)	2.4 (Wicken Fen, England, TL562705)
Extractable P (mg kg^{-1})	2.7 (2.4)	0.2 (Doagh Lough, Ireland, H079526)	7.8 (Lough Corrib, Ireland, M170434)
Exchangeable K^+ (mg kg^{-1})	119 (107)	18 (Lough Talt, Ireland, G397161)	529 (Giant's Causeway, Ireland, C944445)
Exchangeable Ca^{2+} (mg kg^{-1})	3185 (3583)	248 (Mambury Moor, England, SS385171)	12112 (Bleach Lough, Ireland, R441557)
Organic matter (%)	31 (26)	6 (Kenfig, Wales, SS784816)	87 (Lough Corrib, Ireland, M170434)
pH	5.2 (0.5)	4.5 (Rans Wood, England, SU362031)	6.1 (Lough Bunny, Ireland, R382979)

For each of the 22 sites, five topsoil samples (depth 15 cm, diameter 3 cm) were taken with an auger and air-dried. pH was determined electrometrically after mixing air-dried soil with distilled water. Organic matter was determined using loss on ignition (2 h at 800 °C). Total (Kjeldahl) nitrogen was determined using the Kjeltec system 1002 (Tecator, Sweden) and extractable phosphorus using Olsen's method (Allen *et al.* 1989). Calcium was extracted using 1.0 M ammonium acetate with lanthanum chloride, and potassium with 1 M ammonium nitrate; these elements were then determined using air-acetylene flame absorption in an atomic absorption spectrophotometer (Varian Spectr AA 50, Varian, UK).

unique to Ireland, the characteristic species being *Schoenus nigricans, Cirsium dissectum, Anagallis tenella* and *Hydrocotyle vulgaris*. White & Doyle (1982) describe the association as widespread throughout Ireland and Ivimey-Cook & Proctor (1966) found it to be the most widespread and characteristic fen type in the Burren, especially in the low-lying limestone country in the eastern part of the area. The most constant species were: *Agrostis stolonifera, Carex hostiana, Carex panicea, Cirsium dissectum, Mentha aquatica, Molinia caerulea, Potentilla erecta, Schoenus nigricans, Succisa pratensis, Aneura pinguis, Campylium stellatum, Drepanocladus revolvens sensu lato, Fissidens adianthoides* and *Scorpidium scorpioides*. O'Criodain & Doyle (1997) do not support the Cirsio dissecti-Schoenetum nigricantis association as defined by Braun-Blanquet & Tüxen (Ir. Pfl.) and place this community in Ireland within the Schoenetum nigricantis Allorge 1922. They define a new sub association, the Cirsietosum dissecti, that comprises vegetation from the driest of habitats for *Schoenus nigricans*.

In Ireland, *Cirsium dissectum* is sometimes found on the edges of turloughs, growing with *Carex panicea, Carex hostiana, Carex flacca, Molinia caerulea* and *Succisa pratensis* on nutrient poor fens, often on skeletal limestone, or with *Schoenus nigricans, Molinia caerulea, Achillea ptarmica* and *Parnassia palustris* in areas where a layer of fen peat is usually present (Goodwillie 2003); it is not, however, a characteristic turlough species.

de Vere (2007) surveyed 11 sites in England and Wales to examine the range of communities in which *C. dissectum* was found. Sites were chosen that appeared to represent the greatest amount of variation in community type. Ten 2 × 2 m quadrats were surveyed at each site during July or August, and MAVIS Plot Analyser v. 1 (Smart 2000) and Rodwell (1991, 1995, 2000) used to assign the sites to National Vegetation Classification (NVC) communities (Table 2). Detrended Correspondance Analysis (DCA) was carried out (Fig. 3) to examine the relationships between quadrats and sites using the program DECORANA within the Community Analysis Package 2.15 (Pisces Conservation, 2003). Three broad groups emerged when all 11 sites were included in the analysis: (i) SD14b and SD14d (*Salix repens–Campylium stellatum* dune-slack, *Rubus caesius–Galium palustre* and *Festuca rubra* subcommunities), (ii) S24c (*Phragmites australis–Peucedanum palustre* tall-herb fen, *Symphytum officinale* subcommunity) and (iii) the remainder of sites that consisted of M16b (*Erica tetralix–Sphagnum compactum* wet heath, *Succisa pratensis–Carex panicea* subcommunity) and M24c (*Molinia caerulea–Cirsium dissectum* fen meadow, *Juncus acutiflorus–Erica tetralix* subcommunity). When the dune-slack and tall-herb fen communities were removed, the M16b and M24c sites showed some differentiation but still overlapped within the ordination plot. Rodwell (1991, 1995, 2000) does not include *Cirsium dissectum* within the SD14 community and, in addition to the M24, M16 and S24 communities, includes *C. dissectum* within the M21 *Narthecium ossifragum–Sphagnum papillosum* valley mire, M22 *Juncus subnodulosus-Cirsium palustre* fen-meadow, M13 *Schoenus nigricans-Juncus subnodulosus* mire and M29 *Hypericum elodes-Potamogeton polygonifolius* soakway communities and subcommunities.

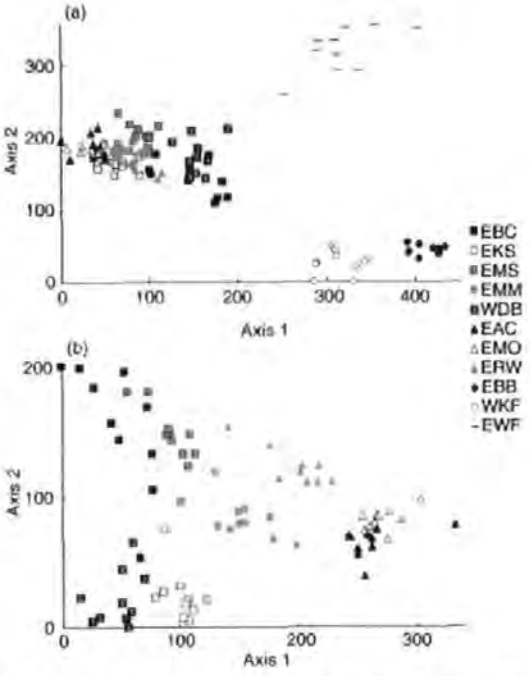

Fig. 3 Detrended Correspondence Analysis of sites where *Cirsium dissectum* is present. Each point represents a single quadrat. See Table 2 for a description of the 3 letter site codes shown. The shape of each point represents a National Vegetation Classification community: squares M24c; squares containing a star M24; triangles M16b; closed circles SD14b; open circles SD14d; dashes S24c. (a) Analysis of all 11 sites surveyed. (b) The same analysis with the SD14b, SD14d and S24c communities removed.

Blackstock *et al.* (1998) surveyed 50 wet grassland sites in lowland Wales and examined edaphic and floristic characteristics within them. They suggested an additional subcommunity called the Welsh nodum (M24x) within the M24 community to cover stands that had a poor representation of preferentials for the existing subcommunity types. Three variants of M24x were described with *C. dissectum* occurring in two of these. When Yeo *et al.* (1998) surveyed 114 remnant stands of neutral and acidic dry grasslands and wet pastures in mid-Wales, *Cirsium dissectum* was most frequent in M24b *Molinia caerulea–Cirsium dissectum* fen meadow, typical subcommunity, M24c and M24x, but small populations were also recorded from a range of other community types.

IV. Response to biotic factors

(A) GRAZING

Cirsium dissectum has soft prickles on its leaves but these do not form an effective grazing deterrent;

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Table 2 Species frequency and abundance data for 11 sites containing *Cirsium dissectum* in Britain

Site Code NVC Community	EBC M24c	EKS M24c	EMS M24c	EMM M24c	WDB M24	EAC M16b	EMO M16b	ERW M16b	EBB SD14b	WKF SD14d	EWF S24c
Achillea ptarmica	V (1–4)	I (1)	IV (1)	II (1)	–	–	–	–	–	–	–
Agrostis canina canina	V (5–9)	V (2–8)	V (3–6)	V (4–6)	I (1–4)	–	IV (1–2)	V (4–8)	–	–	–
Agrostis capillaris	III (2–8)	–	–	–	–	–	–	II (1–4)	–	–	–
Agrostis curtisii	–	–	–	–	–	III (2–8)	V (1–7)	IV (4–5)	–	–	–
Agrostis gigantea	–	–	II (1–8)	–	–	–	–	–	–	–	–
Agrostis stolonifera	II (2–6)	–	–	–	III (1–7)	–	–	–	V (8–9)	IV (1–4)	III (4–7)
Anagallis tenella	–	I (1)	–	–	I (1)	–	I (1)	–	–	III (1–3)	–
Angelica xylvestris	–	–	–	–	I (1)	–	–	–	–	–	IV (1–6)
Anthoxanthum odoratum	I (1)	III (1–3)	III (1)	III (1–2)	IV (1–6)	–	–	–	–	I (1)	–
Asperula cynanchica	II (1–4)	–	–	–	V (1–4)	–	I (1)	I (1)	IV (1)	IV (1–3)	–
Betula pendula seedling	III (1)	–	–	–	–	–	–	II (1)	–	–	–
Betula pubescens seedling	–	–	–	–	–	I (1)	I (1)	–	–	–	–
Briza media	–	–	–	–	I (1)	–	–	–	–	IV (1–8)	–
Calluna vulgaris	I (1)	I (1)	I (2)	III (2–5)	–	V (4–7)	V (2–6)	IV (1–5)	–	–	–
Calystegia sepium	–	–	–	–	–	–	–	–	–	–	II (1)
Carex viridula oedocarpa	–	–	I (1)	IV (1)	–	II (3–4)	V (1–5)	V (1–7)	–	–	–
Carex echinata	–	V (1–3)	I (1)	–	–	–	–	–	–	–	–
Carex flacca	–	–	II (1–5)	I (1–2)	III (1)	–	–	–	–	I (1)	–
Carex hostiana	–	–	–	IV (1–5)	IV (1–4)	–	–	–	–	–	–
Carex nigra	I (1)	–	–	–	II (1)	–	–	–	V (2–8)	IV (1–8)	–
Carex ovalis	III (1–5)	–	–	–	–	–	–	–	–	–	–
Carex panicea	III (1–5)	V (3–5)	IV (1–5)	V (4–7)	IV (1–5)	IV (1–5)	V (3–7)	V (1–6)	III (2–3)	V (1–6)	IV (1–8)
Carex pulicaris	–	I (1)	–	IV (1)	IV (1–3)	I (1)	I (1)	–	–	–	–
Centaurea nigra	–	–	–	–	II (1)	–	–	–	–	I (1)	–
Cirsium dissectum	IV (4–9)	V (1–6)	V (1–6)	V (3–8)	V (1–8)	IV (1–4)	V (3–5)	V (2–6)	III (3–9)	V (3–8)	II (1–5)
Cirsium palustre	II (1–4)	II (1–5)	V (1–5)	I (1)	IV (1)	–	–	–	–	I (4)	III (1–5)
Cynosurus cristatus	–	–	–	–	II (1)	–	–	–	–	–	–
Dactylorhiza maculata	–	IV (1)	–	V (1)	II (1)	–	–	–	–	–	–
Dactylorhiza sp.	–	–	–	–	–	–	–	–	I (1)	II (1)	–
Danthonia decumbens	I (2)	IV (1–3)	–	V (1–3)	III (1–5)	–	V (1–3)	V (1–4)	–	I (1–3)	–
Deschampsia cespitosa	–	–	–	–	–	–	–	–	–	–	I (4)
Eleocharis palustris	–	–	–	–	–	–	–	–	–	I (9)	–
Epilobium hirsutum	–	–	–	–	–	–	–	–	–	–	I (1)
Epilobium palustre	I (1)	I (1)	I (1)	–	II (1)	–	–	–	–	–	–
Epipactis palustris	–	–	–	–	–	–	–	–	IV (1–3)	V (1–3)	–
Equisetum palustre	–	–	–	–	II (1)	–	–	–	V (1–3)	IV (1–4)	–
Erica cinerea	–	–	–	–	–	II (1–5)	III (1–4)	I (1)	–	–	–

Table 2 *Continued*

Site Code NVC Community	EBC M24c	EKS M24c	EMS M24c	EMM M24c	WDB M24	EAC M16b	EMO M16b	ERW M16b	EBB SD14b	WKF SD14d	EWF S24c
Erica tetralix	I (1)	V (4–6)	–	IV (4–6)	–	V (4–7)	V (1–7)	II (1–4)	–	–	–
Eriophorum angustifolium	–	–	–	–	–	–	–	–	–	I (1)	–
Euphrasia nemorosa	–	–	–	–	–	–	–	–	–	I (1)	–
Festuca rubra	–	IV (4–7)	–	–	V (1–8)	–	–	–	–	–	–
Filipendula ulmaria	–	–	–	–	II (1–4)	–	–	–	I (1)	–	V (1–7)
Fragaria vesca	–	–	–	–	–	–	–	–	–	I (1)	–
Fraxinus excelsior seedling	–	–	–	–	I (1)	–	–	–	–	–	–
Galium uliginosum	–	–	–	–	–	–	–	–	–	–	III (1–4)
Holcus lanatus	IV (1–8)	IV (1–4)	III (1–2)	II (1–3)	V (1–6)	–	–	–	I (1)	III (1–3)	I (1)
Hydrocotyle vulgaris	I (4)	II (1–3)	–	–	–	–	–	I (3)	V (1–9)	V (4–8)	III (3–7)
Iris pseudoacorus	–	–	–	–	–	–	–	–	–	–	IV (1–2)
Juncus acutiflorus	IV (1–7)	V (1–8)	IV (1–4)	III (1–8)	V (1–8)	II (1–3)	–	II (1–6)	–	III (1–4)	–
Juncus articulatus	–	–	–	–	–	–	–	–	II (1)	–	–
Juncus conglomeratus	–	III (1–5)	III (1–7)	IV (1–5)	III (1–8)	III (2–8)	–	–	–	–	–
Juncus effusus	I (1)	I (1)	V (1–7)	I (5–7)	–	II (1)	–	–	–	–	–
Juncus squarrosus	–	–	–	–	–	–	III (1–4)	–	–	–	–
Juncus submodulosus	–	–	–	–	–	–	–	–	–	–	V (3–9)
Lactuca serriola	–	–	–	–	–	–	–	–	–	I (1)	–
Lathyrus palustris	–	–	–	–	–	–	–	–	–	–	I (1–3)
Lathyrus sp.	–	–	I (1)	–	–	–	I (1)	–	–	–	–
Leontodon autumnalis	–	–	–	–	–	–	–	–	–	II (1–2)	–
Leontodon hispidus	–	–	–	–	–	–	–	–	–	I (2)	–
Leontodon saxatilis	I (2)	–	–	–	–	–	–	–	–	–	–
Lotus corniculatus	–	–	–	–	–	–	–	–	IV (1–4)	IV (1–4)	–
Lotus pedunculatus	V (1–9)	II (1–4)	V (2–6)	IV (1–3)	V (1–4)	–	–	–	–	–	–
Luzula multiflora	I (4)	II (1–3)	I (1)	III (1)	IV (1–3)	–	–	I (1)	–	–	–
Lychnis flos-cuculi	–	–	–	–	III (1)	–	–	–	–	I (1)	I (1)
Lycopus europaeus	I (1)	–	–	–	–	–	–	–	–	I (1)	–
Lysimachia vulgaris	–	–	–	–	–	–	–	–	–	–	III (1–5)
Lythrum salicaria	–	–	–	–	–	–	–	–	–	–	III (1–4)
Melilotus officinalis	–	–	–	–	–	–	–	–	V (1–7)	–	–
Mentha aquatica	II (1–4)	–	–	–	–	–	–	–	V (1–5)	IV (1–4)	II (1–3)
Molinia caerulea	V (4–7)	V (9–9)	V (8–9)	V (7–9)	V (7–9)	V (6–9)	V (6–9)	V (5–8)	–	III (4–5)	IV (7–9)
Myrica gale	–	–	–	–	–	–	–	I (6)	–	–	–
Narthecium ossifragum	–	V (3–8)	–	–	–	–	–	–	–	–	–
Odontites vernus	–	–	–	–	–	–	–	–	I (1)	–	–
Oenanthe lachenalii	–	–	–	–	–	–	–	–	V (1–3)	–	–

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Table 2 *Continued*

Site Code NVC Community	EBC M24c	EKS M24c	EMS M24c	EMM M24c	WDB M24	EAC M16b	EMO M16b	ERW M16b	EBB SD14b	WKF SD14d	EWF S24c
Ophioglossum vulgatum	–	–	–	–	–	–	–	–	I (1)	–	–
Parentucellia viscosa	–	–	–	–	–	–	–	–	II (1–2)	–	–
Pedicularis palustris	I (1)	–	–	–	–	–	–	–	–	–	–
Pedicularis sylvatica	–	–	–	–	–	–	IV (1–3)	–	–	–	–
Phragmites australis	–	–	–	–	–	–	–	–	–	–	V (1–7)
Plantago lanceolata	–	–	III (1–3)	–	I (1)	–	–	–	I (1)	III (1–7)	–
Plantago major	I (1)	–	–	–	–	–	–	–	–	–	–
Polygala serpyllifolia	–	I (1)	–	–	–	–	III (1)	–	–	–	–
Polygala vulgaris	–	–	–	–	–	–	–	–	–	I (1)	–
Polytrichum sp.	–	–	I (1)	–	–	–	–	–	–	–	–
Potentilla anserina	–	–	–	–	–	–	–	–	V (6–9)	II (3–5)	–
Potentilla erecta	IV (1–5)	V (2–4)	V (1–4)	V (2–4)	IV (1–4)	V (1–4)	V (1–4)	V (1–4)	–	–	–
Potentilla reptans	–	–	–	–	–	–	–	–	V (1–8)	–	–
Prunella vulgaris	II (1–4)	I (1)	I (1)	–	II (1)	–	–	I (4)	–	I (1)	–
Pteridium aquilinum	–	–	–	–	–	I (7)	–	I (1)	–	–	–
Pulicaria dysenterica	–	–	–	–	–	–	–	–	III (1)	V (1–8)	–
Pyrola rotundifolia	–	–	–	–	–	–	–	–	–	I (1)	–
Quercus robur seedling	–	–	II (1)	–	I (1)	–	–	–	–	–	–
Ranunculus acris	–	I (1–2)	–	–	V (1)	–	–	–	V (1–3)	V (1–4)	–
Ranunculus flammula	IV (2–8)	–	I (1)	–	IV (1–4)	–	I (1)	–	V (1–3)	V (1–5)	–
Ranunculus repens	III (1–6)	–	III (1)	–	–	–	–	–	–	–	–
Rhinanthus minor	–	–	–	–	–	–	–	–	–	III (1–3)	–
Rubus caesius	–	–	–	–	–	–	–	–	–	I (1)	–
Rubus fruticosus agg.	I (1)	–	–	–	–	–	I (2)	I (1)	–	–	–
Rumex acetosa	–	I (1)	III (1–2)	–	III (1)	–	–	–	–	–	II (2)
Sagina sp.	–	–	–	–	–	–	I (1)	–	–	–	–
Salix repens	III (1)	–	–	I (1)	–	IV (1–4)	II (1–3)	V (1–5)	II (5–8)	V (1–7)	–
Scutellaria galericulata	–	–	–	–	–	–	–	–	–	I (1)	–
Scutellaria minor	II (1–2)	III (1–3)	I (1)	IV (1–3)	I (1)	–	–	–	–	–	–
Senecio jacobaea	I (1)	–	–	–	–	–	–	–	–	–	–
Serratula tinctoria	–	I (1–4)	III (1–2)	V (1–3)	–	–	II (1)	I (1)	–	–	–
Sphagnum sp.	–	V (8–8)	IV (3–5)	V (3–6)	I (6–8)	II (1–5)	–	–	–	–	–
Stachys palustris	–	–	–	–	–	–	–	–	–	–	II (1)
Succisa pratensis	II (1–7)	V (2–5)	V (1–6)	IV (2–6)	IV (1–5)	–	II (1)	IV (1–4)	–	–	I (1)
Symphytum officinale	–	–	–	–	–	–	–	–	–	–	IV (1–5)
Taraxacum officinale agg.	I (1)	–	–	–	I (1)	–	–	–	–	–	–
Thalictrum flavum	–	–	–	–	–	–	–	–	–	–	IV (1–5)

Table 2 *Continued*

Site Code NVC Community	EBC M24c	EKS M24c	EMS M24c	EMM M24c	WDB M24	EAC M16b	EMO M16b	ERW M16b	EBB SD14b	WKF SD14d	EWF S24c
Trifolium dubium	–	–	–	–	–	–	–	–	–	I (1)	–
Trifolium fragiferum	–	–	–	–	–	–	–	–	IV (1–5)	III (1–5)	–
Trifolium praense	–	–	–	–	I (1–4)	–	–	–	III (1–5)	V (1–5)	–
Trifolium repens	II (1–4)	–	–	–	II (1–5)	–	–	–	IV (1–5)	II (1)	–
Ulex gallii	–	–	–	II (4–6)	–	V (6–8)	–	–	–	–	–
Ulex minor	–	–	–	–	–	–	V (1–5)	I (1)	–	–	–
Veronica scutellata	I (1)	–	–	–	–	–	–	–	–	–	–
Viola canina	–	–	–	–	–	–	IV (1–2)	–	–	–	–
Viola palustris	–	II (1–2)	–	–	V (1–4)	–	–	–	–	–	–

Ten 2 × 2 m quadrats were surveyed at each of 11 sites; in each quadrat all species were identified and abundance estimated using the Domin scale. Roman numerals indicate species frequency (the number of quadrats a species occurs within): I, 1–20%; II, 21–40%; III, 41–60%; IV, 61–80%; V, 81–100%. The numbers in brackets are the Domin range across the quadrats. Site codes: EKS (Knowstone Moor), EMM (Mambury Moor) and EMS (Meshaw Moor) are rhos pasture sites in Devon; WDB (Drostre Bank) is a rhos pasture in Wales; EAC (Aylesbeare Common) is a heath in Devon and EBC (Baddesley Common), EMO (Marlpitt Oak) and ERW (Rans Wood) are New Forest heaths. EBB (Braunton Burrows) is a dune slack in Devon and WKF (Kenfig) a dune slack in Wales. EWF is within Wicken Fen, Cambridgeshire. Sites were assigned to NVC communities using MAVIS Plot Analyser v. 1 (Rodwell 1991, 1995, 2000; Smart 2000).

defoliated plants are seen frequently in cattle-grazed sites. In a growth-room experiment Ross (1999) discovered that *C. dissectum* is reasonably robust in its ability to withstand defoliation. Defoliated plants (with all of the leaves removed) showed a 35% decrease in root relative growth rate (RGR) and a 63% increase in shoot RGR; this allowed leaf biomass to be replaced in less than 8 weeks. Replacement of the leaves depended on adequate nitrogen supply but was not particularly sensitive to low concentrations of phosphorus.

(B) OTHER PLANTS

Cirsium dissectum is susceptible to being out-competed by plants that are able to increase biomass more rapidly, especially when nutrient levels are increased through the effects of fertiliser addition or natural succession (see section **V(B)** below). In an open greenhouse experiment where *C. dissectum* plants were grown with and without a grass competitor (*Agrostis capillaris*), the below-ground presence of the grass reduced the average biomass of *C. dissectum* by a factor of 5.8 (Jongejans 2004).

V. Responses to the environment

(A) GREGARIOUSNESS

Cirsium dissectum can be locally abundant in sites with suitable conditions. It reproduces vegetatively via long rhizomes and typically forms dense patches within all habitat types. In the British Isles, density varied from 4 rosettes m^{-2} in a Welsh rhos pasture to 24 rosettes m^{-2} in a sand dune slack at Braunton Burrows, Devon. Jongejans (2004) recorded higher densities for plants in the Netherlands: in five grasslands density varied from 18 to 133 rosettes m^{-2}.

Figure 4 illustrates the patches of rosettes found within a small population at Wicken Fen, Cambs. The size of each patch was measured and the genetic identity of 35 plants throughout the population was determined using 8 microsatellite loci. Plants with the same multilocus genotype belong to the same clone. Each patch generally contains more than one multilocus genotype suggesting that patches often contain more than one clone (de Vere 2007).

(B) PERFORMANCE IN VARIOUS HABITATS

Table 3 compares morphological variation in plants growing in three different community types. The differences between the populations are due to phenotypic plasticity and genetic differentiation (de Vere 2007). There is considerable variation in the proportion of plants that flower at different sites; de Vere (2007) showed a significant positive relationship between the proportion of *C. dissectum* rosettes that flower and the mean vegetation height within the community ($r^2 = 0.544$, $\beta = 0.753$, $t = 4.99$, $P < 0.001$).

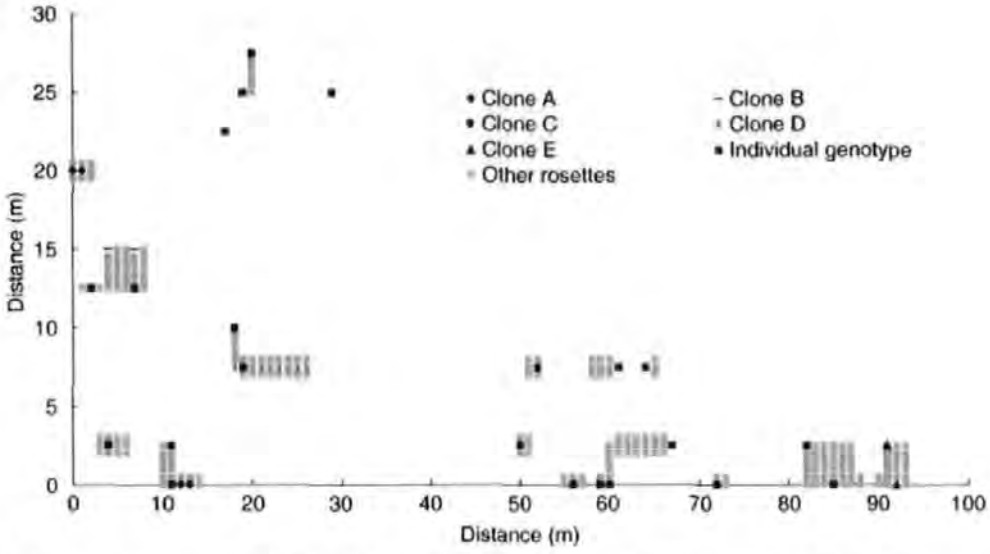

Fig. 4 Schematic representation of a *Cirsium dissectum* population at Wicken Fen, Cambridgeshire. Grey boxes represent the positions of patches of *C. dissectum* rosettes. Black symbols represent individual rosettes that have been genotyped using eight microsatellite loci. Black squares represent rosettes with different multilocus genotypes. Rosettes with the same multilocus genotype are represented with the same symbol (de Vere 2007).

Table 3 Morphological variation in leaves and flowering stems of *Cirsium dissectum* plants from 3 sites in Devon: Knowstone Moor (EKS), Aylesbeare Common (EAC) and Braunton Burrows (EBB). Leaf characters were measured on vegetative rosettes and flowering stem height on flowering rosettes. Means (SD in parentheses) are given, along with the results of one-way ANOVAs followed by *post hoc* Tukey tests. Mean squares, *F*-ratios and *P*-values are shown. Sites that do not share a letter are significantly different

	Knowstone Moor (EKS) ($n = 30$)	Aylesbeare Common (EAC) ($n = 30$)	Braunton Burrows (EBB) ($n = 30$)	MS	*F*	*P*
Number of leaves per rosette	3.1 (1.1)a	3.6 (1.4)ab	3.9 (1.3)b	5.3	3.4	0.037
Leaf length (cm)	10.4 (2.3)a	8.2 (4.1)a	16.2 (6.0)b	514.4	25.7	< 0.001
Leaf width (cm)	2.3 (1.6)ab	1.9 (0.6)a	2.7 (0.7)b	5.3	4.5	0.014
Petiole length (cm)	6.4 (3.3)a	2.7 (2.5)b	5.6 (3.2)a	117.4	13.1	< 0.001
Length of flowering stem (cm)	63.7 (12.0)a	64.9 (24.5)a	50.5 (11.6)b	1896.8	6.3	0.003

Jongejans *et al.* (2006a) examined the effect of increasing productivity on *C. dissectum* in a garden experiment. Individual rosettes of *C. dissectum* were surrounded by *Molinia caerulea* plants and the effect of nutrient enrichment (equivalent of 120 kg N ha^{-1} $year^{-1}$) examined. The biomass of *M. caerulea* tripled in the nutrient-enriched plots and the increased competition caused a decrease in *C. dissectum* survival from 90% in un-enriched plots to 33% in enriched plots. Nutrient enrichment did not increase the total biomass of *C. dissectum* but did increase the percentage of rosettes that flowered from 2.3% in un-enriched plots to 19% in the enriched plots. The turnover rate of plants was also increased, partly due to the increased flowering, but also due to an increase in rosettes that died without flowering. The increased allocation to sexual reproduction in enriched plots was due to the reduced cost of producing flowers and seeds when more nutrients were available and also through a reduction in the root–shoot ratio (Jongejans *et al.* 2006a). The latter is presumably an adaptive response to the need to compete for light rather than nutrients. It therefore appears that in more productive sites, flowering is greater, but *C. dissectum* will become out-competed.

Soons & Heil (2002) investigated the effect of population size and site productivity on the ability of *C. dissectum* to colonize new areas in the Netherlands. Productivity was assessed by clipping three 20 × 20 cm vegetation plots at each site and determining the mean dry mass. Colonization ability consisted of seed production, dispersal ability and germination. Dispersal ability was represented by the relative height that seeds were released (the height of the capitulum above the soil surface minus the height at which the horizontal wind speed was zero) and the terminal velocity of seeds (greater terminal velocity decreased the possibility of long distance dispersal). Smaller populations were found to have lower colonization capacity as they produced fewer seeds per capitulum, had lower percentage germination (under greenhouse conditions) and a narrower range of seed dispersal distances. Sites with

greater productivity had higher seed production and percentage germination was greater under greenhouse conditions. These factors should allow greater colonization capacity of nearby sites but this will only be possible if there are safe sites for seedling establishment; other research suggests that this is very rarely the case (see sections **VI**(C) and **VIII**(D)). Seed dispersal ability decreased with greater productivity, reducing the possibility of longer distance dispersal.

(C) EFFECT OF FROST, DROUGHT, ETC.

Cirsium dissectum is found in moist habitats, although some of its sites such as the heaths of the New Forest, Culm grasslands of south-west England, well-drained *Schoenus* fens on limestone and sand-dune slacks will dry out to an extent during the summer. Ross (1999) showed that plants grown in growth-room conditions were able to survive more than 32 h but less than 64 h in a post-wilting state but that this had a negative impact on relative growth rate (see section **VI**(E)(ii)). It therefore seems likely that *C. dissectum* can survive limited periods of summer drought even though it will affect subsequent growth.

VI. Structure and physiology

(A) MORPHOLOGY

Cirsium dissectum has a hemi-cryptophyte basal rosette growth form. It typically produces new rosettes at the end of long rhizomes but new rosettes can also be formed at the base of existing ones. Rhizomes are a pale straw colour and smooth, with small brown scales at the nodes. Rhizome lengths vary, from close to the parent plant to up to 40 cm, and they grow in a downward curve through the soil, seemingly unaffected by roots or tussocks of other species or soil type (Jongejans 2004). After 2 years, plants have produced a large caudex, approximately 10×2 cm (de Vere 2007). Jongejans (2004) investigated the relationships between rosette size, flowering probability, rhizome formation and site characteristics within five grasslands in the Netherlands. The number of rhizomes produced by a plant varied from 0 to 5 and was generally positively correlated with its caudex weight; caudex weight in turn was positively correlated with site productivity. The most productive grassland had the greatest percentage of rosettes that flowered; flowering rosettes had heavier caudices than vegetative rosettes and produced more rhizomes. Rhizome length and depth did not differ systematically between sites.

(B) MYCORRHIZA

Arbuscular mycorrhizas are found, with the youngest fine roots being the most heavily colonized and older roots having very little detectable arbuscule development (Ross 1999).

(C) PERENNATION: REPRODUCTION

Cirsium dissectum is a long-lived perennial; in productive sites it can flower in its second year at the earliest. Rosettes die after flowering, but generally plants reproduce vegetatively before this.

Survival and growth of seedlings in the field is very rare and clonal propagation is the dominant form of reproduction (Jongejans 2004; Jongejans *et al.* 2006b). In a 5-year study of three grasslands in the Netherlands, Jongejans (2004) found only three seedlings. Similarly Kay & John (1994) and de Vere (2007) found no seedlings in their surveys of populations in the British Isles. The low number of seedlings is caused primarily by very low establishment rates in vegetation stands (Jongejans *et al.* 2006b; de Vere 2007), as seedlings are more abundant in restoration areas where the top soil has been removed near *C. dissectum* populations (Jongejans 2004).

(D) CHROMOSOMES

Material from Port Ellen, Islay was found to be $2n = 34$ (Morton 1977).

(E) PHYSIOLOGICAL DATA

(i) Response to shade

Ross (1999) compared relative growth rate in *C. dissectum* and *Helianthus annuus* at two light levels, 350 and 150 μmol m^{-2} s^{-1} photon flux density, in a growth room with a 16 h day at 22 °C and 15 °C night. Plants were grown in conical flasks containing Rorison nutrient solution. The mean relative growth rate was 0.063 g g^{-1} day^{-1} for *C. dissectum* and 0.125 g g^{-1} day^{-1} for *H. annuus* at the higher light, and 0.055 g g^{-1} day^{-1} for *C. dissectum* and 0.105 g g^{-1} day^{-1} for *H. annuus* at the lower light; Ross (1999) concluded that *C. dissectum* was relatively tolerant of shade. Ellenberg (1988) gave *C. dissectum* a light indicator value of 7, representing a species of well-lit places that also occurs in partial shade, but Hill *et al.* (1999) gave *C. dissectum* within Britain a light indicator value of 8, representing a light-loving plant rarely found where relative illumination in summer is less than 40%.

(ii) Water relations

Ross (1999) investigated water uptake and water-use efficiency using a gravimetric method; plants were grown in conical flasks containing Rorison nutrient solution and the use of water determined by weighing the plants and the conical flasks containing the solutions at regular intervals. *Helianthus annuus* was also grown so that the water use of this mesophytic species could be compared to *C. dissectum*. The experiment was carried out in a growth room with a 16 h day at 22 °C and 15 °C night with day-time light

supplied at 350 µmol m^{-2} s^{-1} photon flux density. The mean relative water uptake (RWU) was 0.41 g mm^{-2} leaf area for *C. dissectum* and 0.28 g mm^{-2} leaf area for *H. annuus*. The mean water use efficiency (measured as dry matter/water used in transpiration) was 0.394 dry mass gain g g^{-1} water used for *C. dissectum* and 0.397 dry mass gain g g^{-1} water used for *H. annuus*. *Cirsium dissectum* thus appears to have a relatively high water use but similar levels of water use efficiency compared to a mesophytic species. Ross (1999) went on to investigate the response of *C. dissectum* to dehydration by growing plants in pots of sand until wilting point was reached and then examining the effects of increasing the number of hours before plants were re-watered. Plants were allowed to reach wilting point (when all leaves were visibly flaccid at the beginning of a day-time cycle); these were then watered for the next 6 weeks to allow a recovery period, and harvested. There was a significant, negative, linear relationship between the number of hours that water was withheld after the wilting point had been reached and subsequent relative growth rate ($r^2 = 0.41$, $P < 0.001$). Plant survival was 100% for plants left for up to 32 h after wilting before being watered but at 64 h all of the plants died (no intervals between were measured).

(iii) Response to nutrients

Pegtel (1983) included *C. dissectum* in a glasshouse pot experiment where plants were watered with nutrient solutions lacking various nutrients. Solutions lacking phosphate and nitrate resulted in plants that showed very little increase in dry mass over time, whilst the absence of sulphate caused little reduction in growth. The absence of potassium slowed down growth after 2 months and symptoms of deficiency became visible as necrotic spots on the leaves. Absence of calcium and magnesium also slowed growth but to a lesser extent than potassium.

Hayati & Proctor (1990) analysed the chemical composition of leaf material collected in June from Aylesbeare Common, Devon, and identified relatively high concentrations of Ca 1.85 (SD 0.41), Mg 0.41 (SD 0.06), K 2.27 (SD 0.19), Na 0.95 (SD 0.21) and P 0.146 (SD 0.071), expressed as percentage dry mass. They also found that plant Ca and Mn was positively correlated with soil Ca and Mn status, while plant Mg and Na were negatively correlated with soil Ca. Hayati & Proctor (1991) investigated plant responses to nutrients (Ca, Mg, N, P and K) added to pots of wet heath peat. This demonstrated that *C. dissectum* showed a strong positive response to calcium carbonate but not to calcium chloride, and to added P but not to N or K.

Cirsium dissectum is not found in very acidic soils and this is likely to be due to toxic effects of raised aluminium and ammonium concentrations in areas with pH lower than 4.5. de Graaf *et al.* (1997) studied the effects of Al concentrations and Al : Ca ratios on the growth of *C. dissectum* in nutrient solution experiments. Aluminium accumulation in the shoots was seen as Al concentrations in the nutrient solutions were increased and this correlated with a reduction in growth at high Al concentrations (200–500 µmol L^{-1}). Poor root development, yellowish leaves and reduced contents of Mg and P in the plants were observed, all indications of Al toxicity. These negative effects were partially counterbalanced when plants were grown in the same Al concentration but with increased Ca concentrations, resulting in lower Al : Ca ratios.

de Graaf *et al.* (1998) investigated ammonium toxicity in a hydroculture experiment using nutrient solutions that differed both in mineral nitrogen form and in ammonium concentration. It was found that plants performed better using nitrate as a nitrogen source than when ammonium was used, with increasing ammonium concentrations causing a reduction in growth. Lucassen *et al.* (2002) elaborated on the findings of de Graaf *et al.* (1998) by suggesting that ammonium as the sole nitrogen source only had a negative effect on *C. dissectum* when in combination with low pH. Ammonium uptake at a rhizosphere pH of 4 resulted in decreased survival rate and biomass development. At higher pH or when nitrate was the sole nitrogen source these effects were not seen. Similarly, Dorland *et al.* (2003) conducted glasshouse dose–response experiments examining the influence of ammonium on germination and survival: a significant negative correlation of both germination and survival with increasing ammonium addition was found at a pH of 4.3.

Franzaring *et al.* (2000) examined the response of *C. dissectum* to elevated ozone concentrations. After 28 days of ozone levels of 26.3 µL L^{-1} (accumulated exposures over a threshold of 40 nL L^{-1}), a significant decrease in root mass and the root:shoot ratio was observed and after 113 days a significant decrease in shoot mass was seen. These results were in marked contrast to *Molinia caerulea*, which showed an increase in growth in response to elevated ozone.

(F) BIOCHEMICAL DATA

No biochemical data are available.

VII. Phenology

The large elliptical–lanceolate leaves of *C. dissectum* die back in winter and are often replaced with much smaller lanceolate leaves. These are hairless and fleshy and persist throughout the winter, often being partially or completely submerged in standing water. The larger, hairy leaves begin to appear again in the British Isles by March with full-sized rosettes present by May. Flowering can start as early as the end of May, with most occurring in June and continuing throughout July. Ripe seed-heads can be found throughout July and August.

VIII. Floral and seed characters

(A) FLORAL BIOLOGY

There are generally 20–160 florets in each capitulum (de Vere 2007). Florets are hermaphrodite; however, Smith (1822) recorded gynodioecy in a population in Ashdown Forest in Sussex but this has not been reported since. The corolla consists of a tube *c.* 9 mm long and a limb of *c.* 11 mm, which divides into 5 irregular lobes of *c.* 5 mm. The corolla tube is white and the limb a deep magenta–purple. Five epipetalous stamens are attached at the junction of the tube and limb of the corolla; the filaments are *c.* 5 mm long and the connate, creamy-white anthers 5.8 mm. The anther tube encloses the central part of the style and ends in five teeth. The style is magenta–purple, *c.* 25 mm (Fig. 5).

The florets have a sweet perfume and produce copious nectar; a range of butterflies, bumblebees and long-tongued dipteran flies visits them. In a small population of *C. dissectum* at Cwm Hydfer, Wales, Kay & John (1994) recorded a mean flight distance between capitula for the small pearl-bordered fritillary (*Boloria selene* Denis & Shiffermueller) of 2.45 m ($n = 7$) with a maximum distance of 4.8 m and a possibility of occasionally much longer interflight distances of greater than 30 m. The common carder bumblebee (*Bombus pascuorum* Scopoli) flew a mean distance between capitula of 1.33 m ($n = 11$) with a maximum of 3 m.

The pollen grain is circular to three-angled in polar view, with a diameter of *c.* 50 μm; it is echinate and circular to slightly elliptical in meridian view (Fig. 6; de Vere 2007).

(B) HYBRIDS

The hybrid *C. dissectum* × *C. palustre* = *C.* × *forsteri* (Sm.) Loudon is not infrequent where the parents occur and is the commonest hybrid thistle (Stace 1997; Preston *et al.* 2002). It is found throughout the range of *C. dissectum* in the British Isles and has been recorded from France and the Netherlands (Hyb. Br. Isl.). It has discontinuously spiny winged, cottony pubescent stems and intermediate leaves and capitula. *Cirsium*

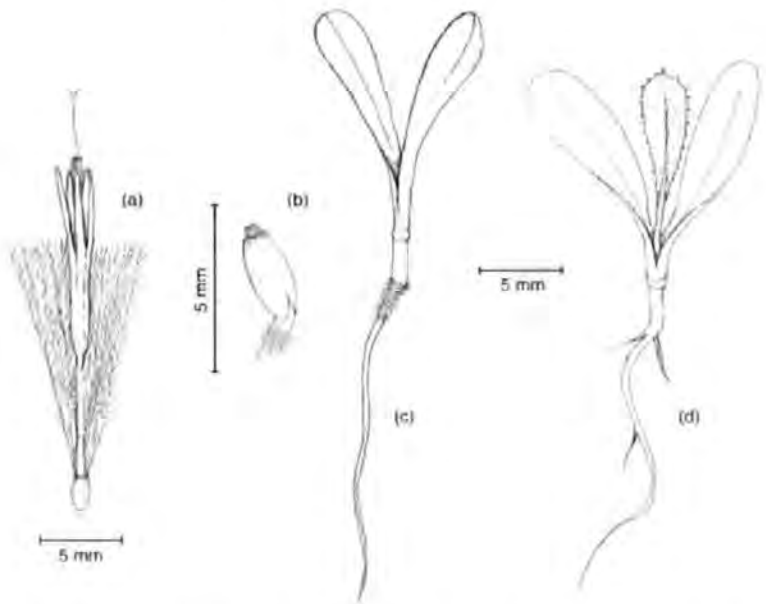

Fig. 5 Floret and seedling development in *Cirsium dissectum*: (a) single floret with part of the pappus removed; (b–d) developing seedling.

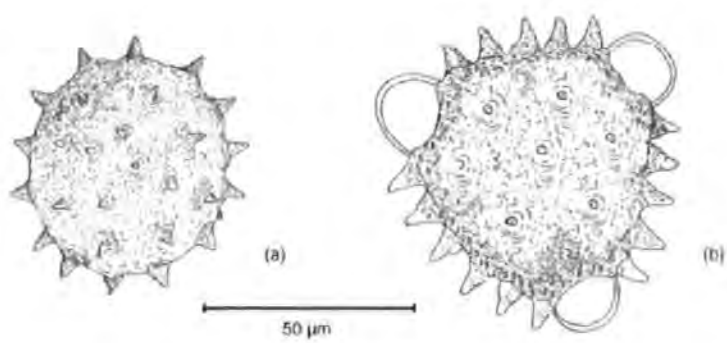

Fig. 6 Pollen grain of *Cirsium dissectum* in (a) meridian and (b) polar view.

dissectum × *C. acaule* = *C.* × *woodwardii* was known from Pen Hill, Swindon, north Wilts between 1848 and 1952 and recorded at South Lopham Fen, east Norfolk, in 1953 (K. J. Walker, pers. comm.).

(C) SEED PRODUCTION AND DISPERSAL

(i) Seed production

Most commonly, only a single capitulum is produced although most populations contain some plants with two or three capitula. *Cirsium dissectum* is self-compatible but selfed capitula produce fewer seeds. Capitula selfed using a paintbrush showed an 89.4% reduction in the number of seeds produced compared to individuals that were out-crossed using the same method ($n = 119$; de Vere 2007). Within each seed head a number of hollow seeds without embryos is often found alongside those that are filled. de Vere (2007) collected 30 seed heads from each of 22 populations throughout the British Isles to examine seed production. The mean number of seeds within a seed head was highly variable both within and between populations. The lowest mean number of seeds per capitulum was observed to be 6.5 (SD 8.5) at Kenfig Burrows in Wales. The highest was at Aylesbeare Common, Devon, with 82.7 (SD 28.1). The mean air-dry mass per achene (with the pappus removed) varied from 1.32 mg (SD 0.42) at a heathland site in the New Forest to 3.63 (SD 0.99) within a highly productive *Schoenus nigricans* fen on the shores of Lough Corrib, Ireland. The mean air-dry achene mass per population was correlated to the concentration of phosphorus in the soil ($r = 0.42$; $P < 0.05$). Jongejans *et al.* (2006b) recorded a range of 72–94 flowers per capitulum with 0.09–0.49 seeds per flower in unpredated capitula and 0–0.37 seeds per flower in predated capitula in three grasslands within the Netherlands. Flowering and seed production varied significantly over the 5 years that the grasslands were monitored.

(ii) Seed dispersal

The pappus often becomes detached before the seed is shed from the capitulum. Wind dispersal occurs in dry conditions as the pappi stick together when they are wet. Rosettes die after flowering and the flowering stem generally dries out and thins just below the capitulum causing the stem to bend over and eventually break, releasing the capitulum often close to the parent plant. This appears to be an additional dispersal mechanism for the seeds still trapped inside.

de Vere (2007) measured the distance travelled by seeds with pappus attached over 2 days at Braunton Burrows, Devon. On both days wind speed was approximately 7–8 m s^{-1} at 10 m height, with occasional stronger gusts. Capitula containing ripe seeds ready for dispersal were located and seeds with pappus attached gently dislodged and tracked. Most of the 110 seeds landed within a few metres of the parent plant with only one seed travelling over 20 m (Fig. 7). Kay & John (1994) investigated seed dispersal at Kenfig, Wales and observed dispersal distances up to 10 m at wind speeds of approximately 2 m s^{-1} and up to 20 m at speeds of around 3–4 m s^{-1}.

Simulated seed dispersal kernels determined by Soons *et al.* (2005) show high probabilities of dispersal close to the parent plant, with dispersal probability dropping to zero at approximately 13 m. The wind speeds used in the model represented the average wind speed distribution during the dispersal season (June to October) in the interior of the Netherlands. Simulations

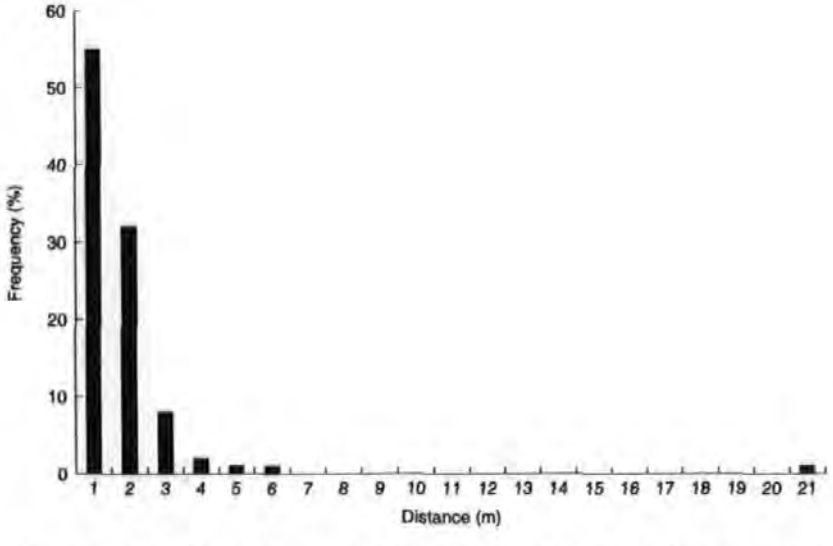

Fig. 7 Dispersal distances for 110 seeds from 11 capitula of *Cirsium dissectum*. Ripe seeds with pappus attached were dislodged from capitula and tracked. If after landing the seed did not move for 2 min, the distance it had travelled was measured. Seed dispersal was measured over 2 days at Braunton Burrows, Devon, with a wind speed of approximately 7–8 m s^{-1} and occasional stronger gusts.

estimated that 1 in 10 000 seeds would be dispersed over 3.4 km under stormy conditions, with an average horizontal wind speed of 22 m s^{-1} at 10 m height) (Soons 2006). The dispersal potential of *C. dissectum* therefore appears to be lower than that of *C. vulgare* (Klinkhamer *et al.* 1988) and *C. erlophorum* (Tofts 1999).

(D) VIABILITY OF SEEDS: GERMINATION

J. Ross (unpublished data) investigated germination at 16, 23 and 31 °C for stratified (moist conditions at 5 °C for 6 weeks) and non-stratified (stored dry at 20 °C) seeds in growth conditions of a 16-h day at 43 µmol m^{-2} s^{-1} photon flux density and an 8-h night. Fifty seeds per treatment were germinated in Petri dishes lined with moist filter paper with each treatment having three replicates (Fig. 8). The final percentage germination was higher and the time taken for half of the seeds to germinate (t50) was lower when the temperature was increased. In all cases stratified seeds germinated better and faster than non-stratified seeds. Seeds were able to germinate in light and dark conditions.

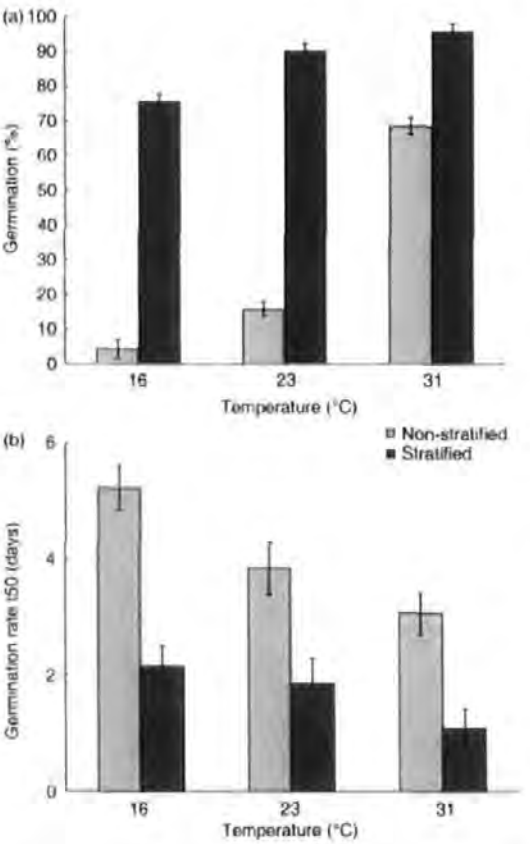

Fig. 8 Germination of stratified and non-stratified seeds of *Cirsium dissectum*. (a) Final percentage germination; (b) germination rate expressed as time taken for 50% of the seeds to germinate (t50). Stratified seeds were stored under moist conditions at 5 °C for 6 weeks, whilst non-stratified seeds were stored dry at 20 °C. Seeds were germinated in a growth room with a 16-h day at 43 µmol m^{-2} s^{-1} photon flux density and an 8-h night. Three replicates of 50 seeds were used per treatment. Germination was carried out in Petri dishes lined with moist filter paper and monitored for 30 days. Adapted from unpublished data of J. Ross.

Isselstein *et al.* (2002) investigated seedling establishment by adding *C. dissectum* seeds to a Cirsio-Molinietum and a species-poor grassland under treatments including irrigation, cutting of the surrounding vegetation and disturbance of the soil surface. Seedling establishment of *C. dissectum* was consistently higher on the Cirsio-Molinietum compared to the species-poor grassland but was still only 15% in the absence of any treatments. Disturbance of the soil and removal of the surrounding vegetation both significantly increased establishment levels. Jongejans *et al.* (2006b) and Soons *et al.* (2005) observed even lower seedling establishment after seed addition in a range of natural Cirsio-Molinietum grasslands within the Netherlands, although again establishment was higher in sites where topsoil had been removed as a restoration measure. Smulders *et al.* (2000) recorded seedling establishment of 9–19% when *C. dissectum* seed was added to experimental plots at a restoration site in the Netherlands.

Germination is thus able to occur over a wide range of temperatures and conditions but seedling establishment in experimental field conditions where seeds are added is low and natural establishment is very rarely observed.

(E) SEEDLING MORPHOLOGY

Stages in seedling development are shown in Fig. 5. Cotyledons are obovate with the tip rounded, 12–35 × 5–8 mm. The first true leaves are elliptical–lanceolate to spathulate, having some hairs above and soft prickles, cottony below.

IX. Herbivory and disease

(A) ANIMAL FEEDERS OR PARASITES

Table 4 lists the animal feeders and parasites recorded from *C. dissectum*. de Vere (2007) collected 30 seed heads from 22 populations throughout the British Isles and observed seed predation by Tephritid flies in 13 of these populations. The effects varied from small holes created in some seeds whilst the rest of the seeds in the capitulum were unaffected, to the complete destruction of all the developing seeds. The highest levels of predation were recorded at Lough Bunny in the Burren, Ireland, with 63% of capitula showing some signs of predation by *Chaetostomella cylindrica* (Robineau-Desvoidy). *Chaetostomella cylindrica* was the most widespread species, occurring in seed heads throughout the British Isles, *Terellia ruficauda* (Fabricius) and *Terellia serratulae* (L.) were found only in seed heads from England and Wales, whilst *Tephritis conura* (Loew) was found only in seed heads from Ireland. Floral herbivory was estimated to reduce seed production by 5% in the Netherlands (Jongejans *et al.* 2006b).

Table 4 Invertebrate species recorded from *Cirsium dissectum*

Species	Source	Ecological notes
Araneae		
Philodromidae		
Tibellus oblongus (Walckenaer)	6b	Nesting in capitulum
Coleoptera		
Curculionidae		
Rhinocyllus conicus (Froehlich)	4	Larvae and adults phytophagous, coastal
Sitona sp.	6a	
Diptera		
Agromyzidae		
Phytomyza autumnalis Griffiths	1	Larvae oligophagous. May house puparium. Mining
Liriomyza strigata (Meigen)	1	Larvae polyphagous. Mining
Tephritidae		
Chaetostomella cylindrica (Robineau-Desvoidy)	2, 6a	Larvae feed and pupate within the capitulum
Terellia ruficauda (Fabricius)	2, 6a	Larvae feed and pupate within the capitulum
Terellia serratulae (Linnaeus)	6a	Larvae feed and pupate within the capitulum
Tephritis conura (Loew)	6a	Larvae feed and pupate within the capitulum
Syrphidae		
Rhingia campestris Meigen	3	Flower visitor
Volucella bombylans (Linnaeus)	3	Flower visitor
Volucella pellucens (Linnaeus)	6b	Flower visitor
Hemiptera		
Cercopidiae		
Philaenus spumarius (Linnaeus)	6b	Nymph sucks sap from flowering stem. Polyphagous
Hymenoptera		
Apidae		
Bombus pascuorum (Scopoli)	3	Flower visitor
Lepidoptera		
Nymphalidae		
Eurodryas aurinia (Rottemburg)	6b	Flower visitor
Boloria selene (Denis & Shiffermueller)	3	Flower visitor
Argynnis aglaja (Linnaeus)	6b	Flower visitor
Pieridae		
Pieris napi Linnaeus	3	Flower visitor
Gonepteryx rhamni Linnaeus	6b	Flower visitor
Papilionidae		
Papilio machaon britannicus (Seitz)	5	Flower visitor
Zygaenidae		
Zygaena trifolii (Esper)	6b	Flower visitor

Sources: 1, Spencer (1972); 2, White (1988); 3, Kay & John (1994); 4, Zwölfer & Harris (1984); 5, Borsje (2005); 6a, N. de Vere, pers. observ.: insects reared from capitula, identified by C. Woolley; 6b, N. de Vere, pers. observ.: insects observed on wild plants.

(B) PLANT PARASITES

Ellis & Ellis (1985) state that the rust *Puccinia calcitrapae* DC. (Basidiomycota: Uredinales) occasionally occurs on the leaves and stem, and the smut *Thecaphora trailii* Cooke (Basidiomycota: Ustilaginales) occasionally affects the flowers, fruits and seeds.

(C) PLANT DISEASES

See section (B) above.

X. History

Cirsium dissectum was first recorded by M. de Lobel in 1576 'Cirsium anglicum ... provenit in pratis C. viri D. Nicolai Pointz equits praefecturae Glostriensis in villa vernacule Acton nominee.' (First Rec.)

XI. Conservation

Cirsium dissectum is generally found in moist, nutrient deficient grasslands and heathlands, habitats that have declined throughout Europe (HMSO 1995; Buck-Sorlin & Weeda 2000). It has been lost from many sites in the British Isles as a result of drainage and succession (Fojt & Harding 1995; Preston *et al.* 2002). *Cirsium dissectum* is listed as Least Concern in the UK by Cheffings *et al.* 2005) but there is a reasonable chance that the UK holds more than 25% of the European population, so may have an international responsibility to protect this species.

In Germany and the Netherlands, drainage, acidification, atmospheric nitrogen deposition, fertilizer use and succession have caused large losses in *C. dissectum*, and remaining sites are often small and fragmented (Buck-Sorlin 1993; Jansen *et al.* 1996; Buck-Sorlin &

Weeda 2000; Jongejans 2004; van den Berg *et al.* 2005). Soons *et al.* (2005) related seed dispersal ability to the availability of suitable habitat within an area of the Netherlands to investigate habitat connectivity. The remaining grasslands containing *C. dissectum* were found to be practically isolated from each other in terms of seed dispersal with the regional survival of the species being completely dependent on a few large populations in nature reserves. Due to these factors, *C. dissectum* is now on the Dutch Red List of endangered species (Rossenaar & Groen 2003; Jongejans *et al.* 2006a).

Research has been conducted in the Netherlands (Berendse *et al.* 1992; Jansen & Roelofs 1996; Jansen *et al.* 1996; Beltman *et al.* 2001) and the UK (Tallowin & Smith 2001) into the best methods for restoring Cirsio-Molinietum fen meadows. Topsoil removal to decrease soil fertility in areas with a suitable hydrological regime has proved to be the most successful approach. The UK Biodiversity Action Plan lists purple moor grass and rush pasture as a priority habitat with plans to recreate this habitat on land adjacent to or nearby existing sites (HMSO 1995) and van Soest (2001) has developed a methodology for the identification of suitable restoration sites in south-west England.

Smulders *et al.* (2000) used AFLP to investigate genetic diversity between source and reintroduced populations of *C. dissectum* in the Netherlands. Source populations showed small but significant genetic differences (Φ_{ST} 0.108). The first generation of reintroduced plants showed less genetic variation than their source populations and were also genetically differentiated, but assignment tests showed that reintroduced populations still resembled their source populations. Calculations showed that reintroduction from more than one source population introduced significantly more genetic variation and Smulders *et al.* (2000) suggested that this might be the best strategy for plants in the Netherlands. Only a small number of populations was studied, however, with a maximum distance between them of 200 km. de Vere (2007) found greater levels of differentiation between populations in the British Isles (G_{ST} 0.276).

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