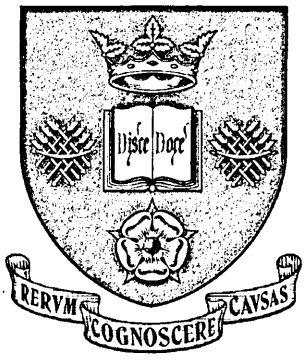

Geographic patterns in the distribution, productivity and population genetic structure of *Cirsium* species across their UK geographic range

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Dedicated to

Claire, Sally and Alice

Summary

Geographically peripheral populations are believed to be particularly important in a species' response to environmental change and for the conservation of intraspecific genetic diversity. It is expected that as a species' range limits are approached, productivity and reproduction will decline and populations will become less abundant and more isolated. Decreased genetic variability and increased genetic divergence of peripheral populations is predicted based on these patterns.

Cirsium heterophyllum reaches a southern geographical limit in the UK, *C. acaule* and *C. eriophorum* reach a northern limit and *C. arvense* occurs throughout the UK. These species have been used to determine whether contemporary patterns of distribution, productivity and reproductive potential across a species' UK latitudinal range are reflected in the predicted patterns of population genetic structure (assessed using microsatellite markers).

Population frequency declines approaching the periphery of *Cirsium acaule* and *C. heterophyllum.* A decline in abundance was found in *C. heterophyllum* only. Community surveys suggest that peripheral populations do not occur in atypical habitat. There is no latitudinal variation in morphological characters across the species range, whereas reproductive potential declines approaching the periphery of the species that reach a latitudinal limit in the UK. Population genetic analysis revealed a decline in genetic variation toward the latitudinal limit of *C. acaule.* This pattern is absent in *C. heterophyllum* despite a marked decline in seed production and increase in population isolation approaching its periphery. *C. heterophyllum* exhibits almost randomised geographical structure of genetic variation.

The lack of agreement between patterns of reproductive potential and population frequency and population genetic structure suggests that contemporary patterns of population distribution and reproduction may be inadequate for indicating patterns of population genetic structure within a species. Interspecific differences in post-glacial history may be important in explaining this disparity.

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Chapter 1: Introduction

1.1.1

It is accepted that climate is the major determinant of plant distribution (Woodward 1996). Species' range boundaries often show a close association with particular climatic variables (Pigott 1968, 1992; Conolly and Dahl 1970; Tofts 1999), an observation that is supported by both correlative approaches linking distributional limits with isometric lines of climate (for example; Salisbury 1926; Conolly and Dahl 1970) and identification of the principal factors that limit the spread of particular species (e.g. Pigott 1968, Pigott and Huntley 1981). Species that show a predictable relationship with particular climatic variables and demonstrate differences in their historic and contemporary distributions have been used as indices of past climatic change (Godwin 1975). Future long-term changes in climate are therefore expected to affect the distribution of many species (Huntley *et al.* 1995; Saetersdal *et al.* 1998; Bakkenes *et al.* 2002).

Evidence from the Quaternary period suggests long term stability in the climatic response of taxa, almost all behaving as though evolutionarily fixed (Bradshaw and McNeilly 1991; Huntley 1991). Major climatic changes in the past were accompanied by plant species migration rather than the mass evolution of tolerant genotypes (Pease *et al.* 1989; Bradshaw and McNeilly 1991; Huntley 1991). In the northern hemisphere most species suffered a series of southward range contractions during the last glacial period, for those surviving glaciation these were followed by rapid northward re-expansion as deglaciation occurred (Comes and Kadereit 1998). This historical evidence suggests that migration is likely to be the most important mechanism by which contemporary species must respond to future changes in global climate.

Populations at the periphery of a species distribution are expected to be particularly important in a species' response to global climate change. Such populations are most likely to be the source of colonists for range expansion (Hewitt 2000). Peripheral populations may include individuals that are pre-adapted to changing environmental

conditions and consequently they are considered important for the future evolutionary diversity of a species (Safriel *et al.* 1994; Lesica and Allendorf 1995), additionally, the periphery of a species' distribution is considered by many to be the most active region of speciation (Lesica and Allendorf 1995 and references therein). Assumptions regarding the genetic structure of peripheral populations often result in them being given high conservation priority (Millar and Libby 1991; Lesica and Allendorf 1995).

Low temperatures may limit poleward spread through their actions on both the vegetative (Woodward 1990; 1997) and reproductive phases of plant growth (Pigott 1968; Pigott and Huntley 1981; Woodward 1990). The factors limiting spread in the equatorial direction are less clear; high temperature (Conolly and Dahl 1970) and water availability (Pigott and Pigott 1993) have been implicated and competitive exclusion may play a key role (Woodward 1996). Many species exhibit a decline in seed production toward the range boundary (Pigott 1968; Pigott and Huntley 1981; Reinartz 1984b; Eckert and Barrett 1993; García *et al.* 2000; Dorken and Eckert 2001). The reproductive phase of the plant lifecycle shows particular sensitivity to climate (Marshall 1968; Pigott 1968, Pigott and Huntley 1981; Houle and Filion 1993; Despland and Houle 1997; Woodward 1997; García *et al.* 2000). Variation in vegetative characters such as plant size (Marshall 1968; Clevering *et al.* 2001) and differential allocation to above and below ground organs (Benowicz *et al.* 2000) have also been reported toward a species' range limit. Differences in plant size may ultimately be reflected in the reproductive success of the plant (Reinartz 1984ab; Primack 1987).

The favourability of a species' environment is presumed to decline from core to peripheral regions of the species geographic range (Brown 1984). This may result in a species occupying atypical habitat in peripheral areas (Lesica and Allendorf 1995) where the impact of decreased environmental favourability is reduced. A decline in the favourability of a species' typical habitat is expected to lead to a reduction both in the abundance of individuals within a population and the number of populations within an area (Hengeveld and Haeck 1982; Brown 1984). An increase in the geographic isolation of peripheral populations compared with those in more central areas of the species' range will result in a decrease in pollen and seed transfer between them (Ellstrand 1992; Ellstrand and Hoffman 1990; van Dorp *et al.* 1996), thereby resulting

in increased genetic isolation. Demographic instability resulting from reduced migration between isolated populations (Schaal and Leverich 1996) and reduced plant regeneration (Pigott 1992) will increase the extinction risk of peripheral populations (Channel and Lomolino 2000).

Low genetic variability has been reported in geographically peripheral populations (Eckert and Barrett 1993; Durka 1999; Lammi *et al.* 1999; Jones *et al.* 2001) and linked with increased risk of population extinction (Newman and Pilson 1997; Channel and Lomolino 2000). Peripheral populations may have reduced genetic variability as a result of both reduced size and increased isolation and demographic instability (Ellstrand and Elam 1993; Raijmann *et al.* 1994; Schaal and Leverich 1996). Genetic isolation may lead to genetic divergence of these populations, both from each other and from populations in more central areas of the species' range (Lesica and Allendorf 1995). Divergent populations may also be particularly valuable for the conservation of a species' genetic diversity (Millar and Libby 1991).

The geographic pattern of genetic structure within a species is often predicted from the contemporary geographic distribution of populations (eg. Lesica and Allendorf 1992, 1995; Ellstrand 1992; Ellstrand and Elam 1993; Schaal and Leverich 1996) and a species' ecological characteristics such as breeding system and floral morphology (Loveless and Hamrick 1984; Hamrick and Godt 1996). Present day patterns of variation in reproductive potential also lead to predictions regarding the genetic structure (Schnabel *et al.* 1998) and persistence of populations (Newman and Pilson 1997). However, some studies reveal a striking lack of agreement between current population distribution and genetic structure (Stone and Sunnucks 1993; Gabrielsen *et al.* 1997; Kropf *et al.* 2002).

Geographic patterns in population genetic structure may result from persistent effects of species' migrations (Stone and Sunnucks 1993; Ibrahim *et al.* 1996; Dumolin-Lapègue *et al.* 1997; Petit *et al.* 1997), emphasising the importance of history in understanding contemporary patterns of genetic variation. History is also important in explaining the current distributional limits of long-lived species (Pigott 1992) since for some plant species there is a disparity between the theoretical distribution based on identified

physiological limits and the realised distribution of current populations (Pigott and Huntley 1981; Woodward 1990).

The postglacial history of a species may be a major force in shaping present day patterns of geographic structure in genetic variation (Comes and Kadereit 1998; Taberlet *et al.* 1998; Hewitt 2000). Putative glacial refugial populations are likely to contain high levels of genetic diversity compared with populations occurring in more recently colonised areas of the species range (Comes and Kadereit 1998 and references therein). Since genetic diversity is expected to decrease with distance from the proposed refugia, clines in genetic diversity across broad geographic areas may indicate the routes of a species' post-glacial migration (Stone and Sunnucks 1993; Dumolin-Lapègue *et al.* 1997; Petit *et al.* 1997; Kropf *et al.* 2002). Post-glacial mixing of previously isolated lineages has the potential to disrupt this pattern, resulting in regions of unusually high diversity where the lineages meet (Abbott *et al.* 2000; Walter and Epperson 2001). Although it is accepted that most post-glacial colonisation of northern Europe occurred from glacial refugia in more southern regions (Pigott and Walters 1954; Godwin 1975; Taberlet *et al.* 1998; Hewitt 2000), there is less agreement regarding possible perglacial persistence of some species in arctic regions (Gabrielsen *et al.* 1997; Tollesfrud *et al.* 1998; Tremblay and Schoen 1999; Abbott *et al.* 2000).

General patterns of post-glacial migration have been identified in Europe and North America by comparing geographic patterns of genetic variation in a range of species (Soltis *et al.* 1997; Taberlet *et al.* 1998; Hewitt 2000). However, close agreement between patterns displayed by individual species is limited, possibly due to species-specific differences in factors such as migration and extinction rates and competitive ability (Comes and Kadereit 1998). The diversity of species' ecological characteristics is reflected in analogous diversity in the palaeobotanical record (West 1970; Godwin 1975), therefore consideration of a species' ecology is also likely be important in interpreting present day patterns of genetic variation within a species.

Populations at the periphery of a species geographic range represent both the present day limit of the species distribution and the historic limits of its' post glacial migration (Hewitt 2000). Given the assumed importance of peripheral populations for the future diversity of a species, the aims of this thesis are twofold:

1) To investigate whether predicted declines in present-day population frequency, plant productivity and reproduction occur approaching the range edge.
2) To determine if such declines are reflected in the predicted parallel decrease in genetic variability and increase in genetic divergence of populations at the edge of the species' geographic range.

This work will investigate four *Cirsium* species (*Cirsium acaule*, *C. arvense*, *C. eriophorum* and *C. heterophyllum*). *Cirsium acaule* and *C. eriophorum* reach a northern distributional limit in the UK, *C. heterophyllum* reaches a southern distributional limit in the UK and *C. arvense* is a widespread species that will be used for comparison. Previous studies have reported a close association between the range northern boundaries of *Cirsium acaule*, *C. eriophorum* and the southern boundary of *C. heterophyllum* and isotherms of summer temperature (Pigott 1968; Conolly and Dahl 1970; Tofts 1999), implicating climate as a major factor in determining the distribution of these species. As this work will investigate potential north-south clines in characters in these species, latitude will be used as a proxy for distance from the core of the species distribution. Latitude is a good proxy for the action of many factors such as radiation balance, length of growing season and the frequency of frost events, Valentini *et al.* (2000) reported latitude to be a better correlate of ecosystem respiration than any single factor they tested (including mean annual temperature, precipitation and elevation) when investigating forest carbon balance. Furthermore, investigating core-periphery patterns in species has the inherent problem that range edges are extremely difficult to define reliably (Blackburn *et al.* 1999). Thus when investigating north-south clines, latitude is a surrogate measure that provides both an indication of the position of populations and the distance between them in the absence of a reliable and repeatable way of defining a species range edge.

Latitudinal patterns in population frequency of these species will be investigated by analysing species distribution data from botanical surveys of the UK, the abundance of individuals within populations will be assessed by direct measurement in the field. Plant community survey data for each population will be used to classify the plant communities in which these *Cirsium* species occur and allow comparison both within and between the species investigated. Community surveys will indicate whether populations occur in atypical communities in any regions of the species' range and

provide an indication of the comparability of data from different populations. Plant productivity and reproduction will be assessed by direct measurement of vegetative characteristics and flower and seed production in populations throughout the UK latitudinal range of each species. Using microsatellite molecular markers developed for this study, population genetic analysis will be employed to determine levels of genetic variation within populations and assess genetic distance between populations throughout the UK latitudinal range of the *Cirsium* species investigated.

To gain a full picture of the phylogeographic structure of these *Cirsium* species it would be necessary to survey populations throughout the range occupied by each species. However, it is not the aim of this work to present a comprehensive study into their phylogeography, rather to determine whether patterns in the distribution, reproduction and productivity of these species are reflected in parallel patterns of population genetic structure (as outlined above). Consequently, with the exception of *C. heterophyllum*, these species were sampled only within their UK range. *Cirsium heterophyllum* however, reaches a southern lowland limit in the UK but occurs at high altitude throughout the mountains of Europe – thus more southerly European populations exist beyond its southern lowland UK limit. Additional populations of this species were surveyed in Switzerland and Italy in an attempt to determine whether any potential decline in genetic variation toward the southern periphery of this species in the UK was a result of a range edge being reached. If this was so then it was expected that the genetic variation in this species southern peripheral region in the UK would be lower than both that in its core lowland region in Scotland and core high altitude regions in more southerly areas of Europe.

There may be major differences between latitudinal patterns reported from the natural environment and those measured in controlled environment and common environment experiments. Clevering *et al.* (2001) found the opposite pattern in relative growth rate with latitude when growing *Phragmitis australis* from a variety of latitudes in a common garden to that observed in the natural environment by Čížková (1999, in Clevering *et al.* 2001). Winn and Gross (1993) reported latitudinal variation in seed weight and flower number of *Prunella vulgaris* when populations were grown in a common environment but these differences were not evident between naturally occuring populations in the field. Likewise, field and common environment results were not

comparable in some of the traits measured by Lacy (1984), Reinartz (1984b) and Newman and Pilson (1997).

Whereas controlled environment and common environment studies are highly informative when attempting to separate the genetic and environmental components of plant variation (see the references listed above), they do not necessarily indicate the pattern of response that will be realised under conditions occurring in the natural environment. Consequently, there is a danger that erroneous predictions may result when the results from one environment are applied to another (Newman and Pilson 1997). Since this investigation aims to determine patterns that are evident across the latitudinal range of species in the natural environment, all data are from measurement of *in situ* populations and material collected from these populations in the field.

1.1.2 Species introduction

Congeneric species were used in this study in order to reduce major interspecific differences that may exist between apparently similar species as a result of their differing long-term evolutionary history (Kelly and Woodward 1996; Silvertown and Dodd 1996; Blackburn and Gaston 1998). Four *Cirsium* species (Family: Asteraceae) were chosen, based on their geographic distribution within the UK, these are detailed below. The nomenclature of all species referred to in this study follows that used by Stace (1997).

Cirsium acaule (L.) Scop.

C. acaule (Fig. 1.1.1) reaches a northern distributional limit in the UK (Fig. 1.2.1). In Europe it extends from northern England and Estonia southwards to southern Spain, Serbia and southeast Russia (Tutin *et al.* 1976). *C. acaule* is a perennial, gynodioecious species. In addition to reproduction by seed, clonal reproduction occurs by the production of new rosettes from the branching rhizome (Pigott 1968). *C. acaule* is confined to closely grazed calcareous pastures (Pigott 1968; Clapham *et al.* 1981).

Cirsium arvense (L.) Scop.

C. arvense (Fig. 1.1.1) is widespread throughout the UK (Fig. 1.2.2). It is absent from some of the mountainous areas of Scotland (Perring and Walters 1990). *C. arvense* occurs almost throughout Europe and is absent only from Svalbard in the extreme north and Crete and the Azores in the south (Tutin *et al.* 1976). *C. arvense* is a perennial species, it is incompletely dioecious as some male flowers produce viable seed. In addition to reproduction by seed, clonal reproduction occurs by the production of new shoots from underground root and stem tissue (Grime *et al.* 1989; Heimann and Cussans 1996). *C. arvense* occurs in a wide variety of disturbed and ruderal habitats such as arable fields, grassland, waste ground, waysides etc (Clapham *et al.* 1981; Grime *et al.* 1989).

Cirsium eriophorum (L.) Scop.

C. eriophorum (Fig. 1.1.1) reaches a northern distributional limit in the UK (Fig. 1.2.3). It is absent or rare in the extreme southeast and southwest of the UK. This species occurs throughout western and central Europe, northwards to northern England and extending to northern Italy and western and southern parts of the Balkan peninsula (Tutin *et al.* 1976). *C. eriophorum* is a monocarpic, hermaphrodite species, normally classified as biennial although it may take longer to complete its life cycle. Reproduction of *C. eriophorum* is entirely by seed (Tofts 1999). *C. eriophorum* occurs on calcareous soil in grasslands, open scrub, waysides and disturbed habitats associated with quarrying (Clapham *et al.* 1981; Tofts 1999).

Cirsium heterophyllum (L.) Hill

C. heterophyllum (Fig. 1.1.1) reaches a southern low altitude distributional limit in the UK (Fig. 1.2.4). It occurs at low altitiudes in northern Europe and the eastern part of the former USSR and mountain ranges of Europe southwards to the Pyrenees and Transylvania (Tutin *et al.* 1976). There appears to be no published information available regarding the breeding system and floral morphology of the perennial *C. heterophyllum.* In addition to reproduction by seed, clonal reproduction occurs in this species by production of new shoots from stolons (Clapham *et al.* 1981). *Cirsium heterophyllum* occurs in upland meadows, grasslands, streamsides, waysides and open woodland (Clapham *et al.* 1981; Rose 1991).

Hybrids

Morphological intermediates, which are probably hybrids, are frequent within the genus *Cirsium* (Tutin *et al.* 1976). *Cirsium acaule* hybridises most readily with *C. tuberosum* producing F_1 hybrids that are intermediate in many features, though successive backcrosses give plants that are not easily distinguished from the pure species. Hybrids of *C. acaule* with *C. vulgare*, *C. palustre* or *C. arvense* occur rarely and are intermediate in morphology; *C. acaule* does not hybridise with *C. eriophorum* (Pigott 1968). Rare hybrids of *C. eriophorum* with *C. vulgare* have been reported in England and with *C. palustre* and *C. arvense* elsewhere in Europe (Tofts 1999). In addition, *Cirsium arvense* is known to hybridise with other European *Cirsium* species (Moore 1975). *Cirsium acaule* hybridises naturally with *C. heterophyllum* in Central Europe but such hybrids do not occur in the UK where their distributions overlap only in Derbyshire (Pigott 1968). A morphological intermediate between *C. heterophyllum* and *C. palustre* was observed at a site in Scotland during the course of this investigation (Site HM2, Table 1.3.1). No other putative *Cirsium* hybrids were observed at any of the other sites visited.

The approximate locations of selected survey regions within mainland UK are shown in Fig. 1.3.1. Accurate site locations for survey populations of each species are given in Table 1.3.1.

Credits: *C. arvense*, A. Voswinkle; all others A. Jump.

Fig. 1.1.1 Cirsium species in flower. *C. acaule* (top left), *C. arvense* (top right), *C. eriophorum* (bottom left), *C. heterophyllum* (bottom right).

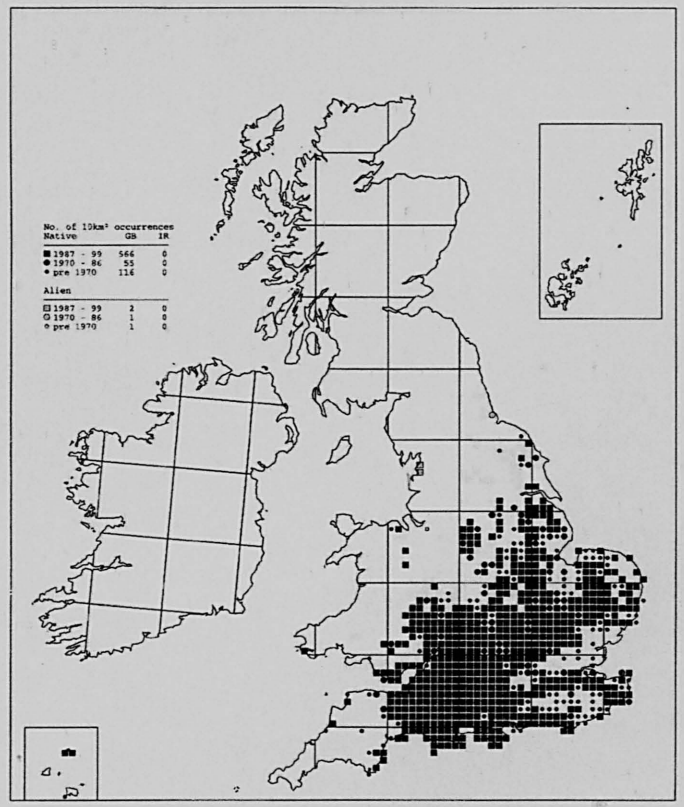

Fig. 1.2.1 UK and Irish distribution map of *Cirsium acaule* showing presence in 10km squares. Reproduced from Preston *et al.* (2002).

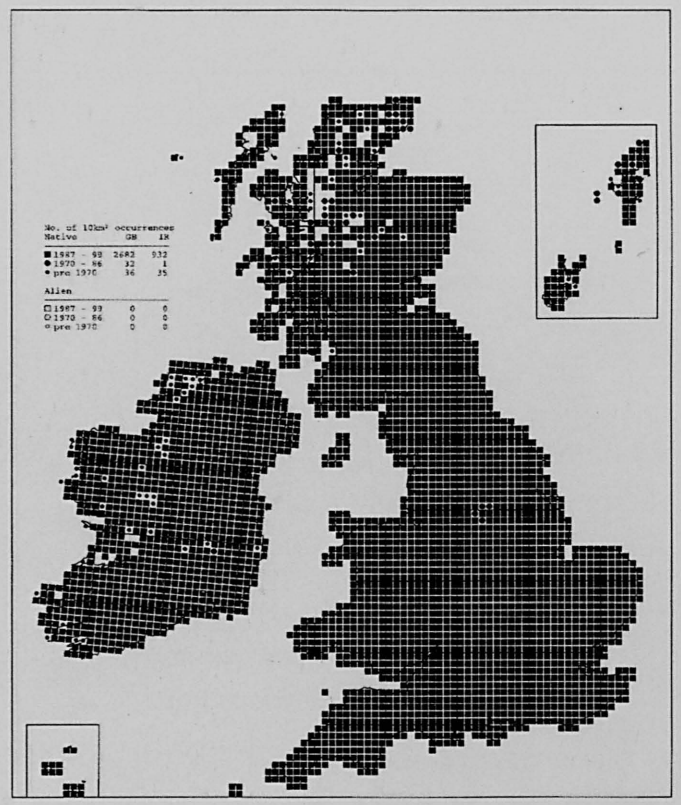

Fig. 1.2.2 UK and Irish distribution map of *Cirsium arvense* showing presence in 10km squares. Reproduced from Preston *et al.* (2002).

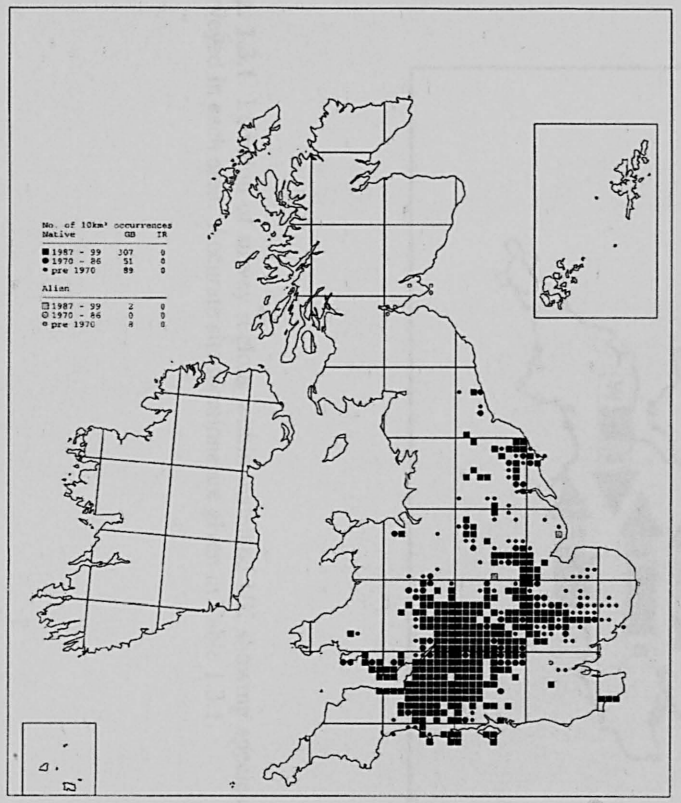

Fig. 1.2.3 UK and Irish distribution map of *Cirsium eriophorum* showing presence in 10km squares. Reproduced from Preston *et al.* (2002).

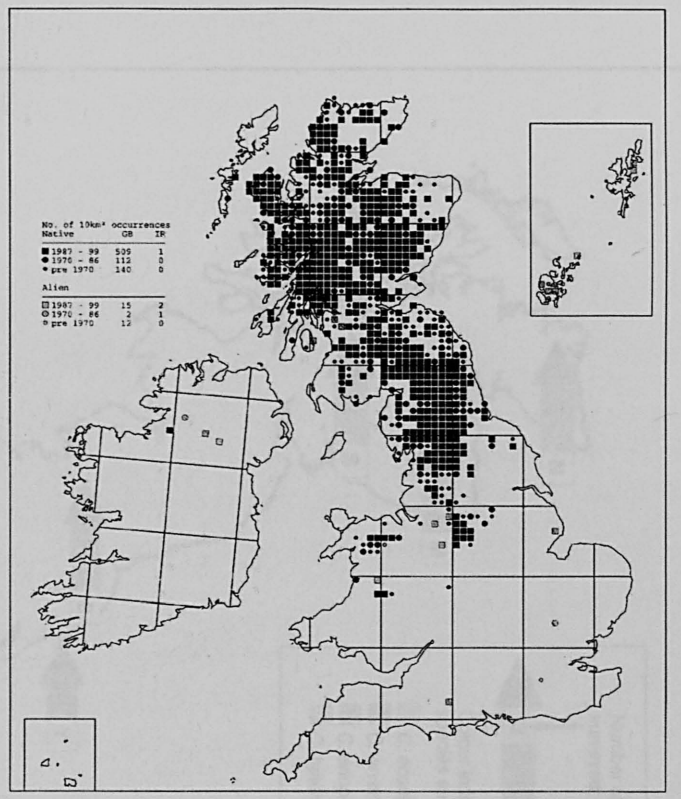

Fig. 1.2.4 UK and Irish distribution map of *Cirsium heterophyllum* showing presence in 10km squares. Reproduced from Preston *et al.* (2002).

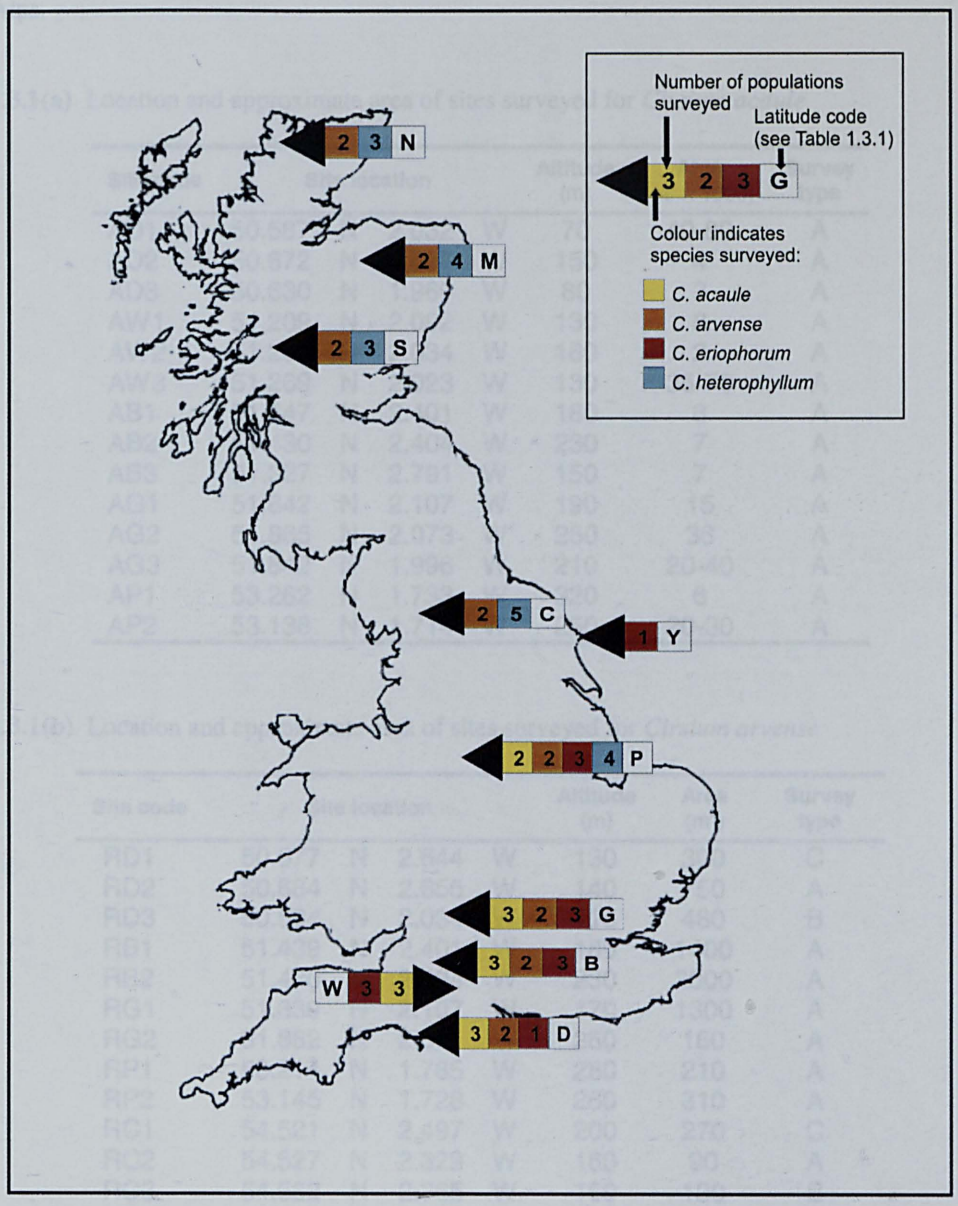

Fig. 1.3.1 Location of survey regions within mainland UK showing species and number of populations surveyed in each area. Accurate site locations are given in Table 1.3.1.

Table 1.3.1(a)-(d) Details of surveyed populations of *C. acaule*, *C. arvense*, *C. eriophorum* and *C. heterophyllum*. With the exception of HA1-HA4, site codes refer to survey areas within the UK (as shown in Fig. 1.3.1); HA1-HA4 refer to populations of *C. heterophyllum* surveyed in the Swiss and Italian Alps.

Table 1.3.1(a) Location and approximate area of sites surveyed for *Cirsium acaule*

Site code	Site location				Altitude (m)	Area ($m^2 \times 1000$)	Survey type
AD1	50.587	N	2.032	W	70	40-80	A
AD2	50.672	N	2.587	W	150	4	A
AD3	50.630	N	1.969	W	80	3	A
AW1	51.209	N	2.092	W	130	8	A
AW2	51.262	N	2.034	W	160	2	A
AW3	51.269	N	2.023	W	130	35-70	A
AB1	51.447	N	2.401	W	180	8	A
AB2	51.430	N	2.404	W	230	7	A
AB3	51.327	N	2.791	W	150	7	A
AG1	51.842	N	2.107	W	190	15	A
AG2	51.865	N	2.073	W	260	36	A
AG3	51.842	N	1.996	W	210	20-40	A
AP1	53.262	N	1.733	W	320	6	A
AP2	53.138	N	1.714	W	250	20-30	A

Table 1.3.1(b) Location and approximate area of sites surveyed for *Cirsium arvense*

Site code	Site location				Altitude (m)	Area (m^2)	Survey type
RD1	50.677	N	2.644	W	130	300	C
RD2	50.684	N	2.655	W	140	750	A
RD3	50.594	N	2.034	W	110	480	B
RB1	51.439	N	2.401	W	180	1400	A
RB2	51.430	N	2.404	W	230	3000	A
RG1	51.839	N	2.107	W	170	1300	A
RG2	51.862	N	2.071	W	260	160	A
RP1	53.214	N	1.765	W	280	210	A
RP2	53.145	N	1.728	W	260	310	A
RC1	54.521	N	2.497	W	200	270	C
RC2	54.527	N	2.329	W	160	90	A
RC3	54.532	N	2.365	W	160	120	B
RS1	56.389	N	5.196	W	120	15	B
RS2	56.364	N	5.183	W	100	40	B
RM1	57.321	N	4.363	W	240	480	C
RM2	57.101	N	3.987	W	260	225	A
RM3	57.005	N	4.170	W	310	180	B
RN1	57.967	N	4.735	W	40	40	B
RN2	58.163	N	4.990	W	90	200	B

Table 1.3.1(c) Location of sites surveyed for *Cirsium eriophorum*

Site code	Site location				Altitude (m)	Survey type
ED1	50.677	N	2.644	W	130	C
EW1	51.209	N	2.093	W	130	C
EW2	51.259	N	2.024	W	150	C
EW3	51.202	N	2.084	W	160	C
EB1	51.441	N	2.401	W	220	C
EB2	51.430	N	2.414	W	220	C
EB3	51.420	N	2.426	W	170	C
EG1	51.847	N	2.102	W	250	C
EG2	51.865	N	2.074	W	260	C
EG3	51.842	N	1.996	W	230	C
EP1	53.256	N	1.664	W	230	C
EP2	53.258	N	1.655	W	300	C
EP3	53.258	N	1.667	W	280	C
EY1	54.257	N	0.465	W	60	C

Table 1.3.1(d) Location and approximate area of sites surveyed for *Cirsium heterophyllum*

Site code	Site location				Altitude (m)	Area (m^2)	Survey type
HA1	46.100	N	7.950	E	1750	40	B
HA2	45.840	N	7.744	E	1690	50	B
HA3	45.836	N	7.746	E	1560	2150	B
HA4	45.944	N	7.733	E	1700	1850	B
HP1	53.214	N	1.765	W	280	50	A
HP2	53.231	N	1.844	W	320	30	A
HP3	53.241	N	1.780	W	290	100	A
HP4	53.166	N	1.879	W	270	860	A
HC1	54.408	N	2.337	W	220	70	A
HC2	54.439	N	2.587	W	170	120	A
HC3	54.862	N	2.508	W	200	100	A
HC4	54.447	N	2.387	W	270	900	A
HC5	54.377	N	2.346	W	300	20	B
HS1	56.490	N	4.748	W	200	200	A
HS2	56.400	N	5.213	W	80	30	A
HS3	56.321	N	3.685	W	100	160	A
HM1	57.101	N	3.987	W	260	150	A
HM2	57.015	N	4.162	W	290	340	A
HM3	57.327	N	3.021	W	320	40	A
HM4	57.420	N	2.627	W	230	50	A
HN1	57.990	N	4.814	W	150	40	A
HN2	58.243	N	5.177	W	50	70	A
HN3	57.753	N	5.011	W	200	30	A

Notes.

Site code: The first letter of the site code signifies the species, the second the latitudinal region in which the population was surveyed.

Survey type: A = population surveyed for both population genetic structure and abundance, community and productivity measures. B = population surveyed for population genetic structure only. C = population surveyed for abundance, community and productivity measures only.

Population area: Estimated by pacing the length and width of the area occupied by the population. For *C. acaule*, population limits were marked on 1:50,000 scale maps and approximate area calculated accordingly.

Chapter 2: Patterns in the abundance and frequency of *Cirsium* species across their UK geographic range

2.1.1 Introduction

Geographical isolation of populations may have profound effects in terms of population structure and demography. Increasing geographical isolation of plant populations is likely to lead to a decrease in both seed dispersal and pollen flow between populations (Ellstrand and Hoffman 1990; van Dorp *et al.* 1996). A population's probability of extinction is directly correlated with its demographic variability and inversely correlated with density and immigration rate (Channel and Lomolino 2000). The relative isolation of populations is thus an important consideration in both conservation strategy and any investigation into patterns of genetic diversity across a species range.

It has long been accepted that there is much variation in the local abundance of individual species across their geographic range. At the coarsest resolution it is held as a general biogeographical rule that the abundance of a species declines from the core areas of its distribution toward the periphery (Hengeveld and Haeck 1982; Brown 1984; Lawton 1993). Numerous studies support this pattern (Hengeveld and Haeck 1982; Brown 1984; Svennson 1992; Brown *et al.* 1995) though there are also many exceptions (Blackburn *et al.* 1999; Pérez-Tris *et al.* 2000; Brewer and Gaston 2002).

Density of populations within an area is expected to decrease toward the periphery of a species range (Brown 1984); this pattern is seen even where there is no observed decline in abundance (Hall *et al.* 1992; Pérez-Tris *et al.* 2000), though this is not an inviolable rule (Brewer and Gaston 2002). A glance through distribution maps such as those presented in the Atlas of the British Flora (Perring and Walters 1990) gives the immediate impression that species distributions tend to become more patchy as a species approaches the limits of it's geographic range. It is important however to distinguish between a decline in typical habitat and a decline in occurrence in typical habitat and hence the measure of occupancy is sometimes used (Svennson 1992).

The most widely cited theory proposed to explain patterns in frequency and abundance is that of Brown (1984). Brown suggested that decline in abundance from core to peripheral range areas could be ascribed to spatial variation in the physical and abiotic variables that make up Hutchinson's (1957) multidimensional niche. Brown's theory assumes that combinations of many physical and biotic variables determine the abundance and distribution of a species and that the probability of sites having similar combinations of environmental variables is an inverse function of the distance between them. Abundance is expected to decline with distance from the core as an increasing number of niche dimensions become less favourable.

Variation in density is described both at the within population level and at the between population level, both of which are investigated here. I shall use *abundance* to describe the density of individuals *within a population* and *frequency* to describe the density of populations *within an area.*

2.1.2 Survey scale and population isolation

The most available distribution maps of organisms found in the UK are those based on 10km × 10km square (hectad) survey data recording presence of species within each square. Whereas this level of detail may be suitable for identifying patterns of species distribution it is outside the scale of most ecological processes such as pollen flow and seed dispersal (Ellstrand and Hoffman 1990; van Dorp *et al.* 1996). Most population processes in plants occur on a considerably smaller scale and thus a hectad based distribution map is not useful for inferring changes in the relative isolation of populations between areas. The hectad survey data are simple presence/absence data and as a consequence, a single individual of a species will lead to a record for the entire hectad. It is possible therefore that the hectad distribution map may simply reflect coarse scale habitat availability without informing on population frequency. Mapping at the hectad scale does not differentiate between scenarios where the frequency of populations on the local scale is similar throughout all areas of a species range as opposed to that where frequency decreases in some areas. This information is particularly important when considering population structure and ecological processes alongside patterns in geographical distribution.

Some areas of the UK have been surveyed for some groups of organisms on a 2km × 2 km square (tetrad) basis, though this is the exception rather than the norm. This resolution is of greater ecological relevance but requires a vast increase in sampling effort. In order to determine whether geographic isolation increases approaching the range limits of the *Cirsium* species investigated here, it was necessary to find some way of using existing surveys to give an indication of population frequency at the local level.

2.1.3 Botanical surveys of the British Isles

The most recent large scale botanical surveys of the British Isles are the Botanical Society of the British Isles (BSBI) Monitoring Scheme of 1987-1988 and the Plant Atlas 2000 project (collected between 1987 and 2001 for the Atlas of Flowering Plants and Ferns of Britain and Ireland; Preston *et al.* 2002).

The BSBI Monitoring Scheme was a sample hectad and tetrad survey of the flora of Britain and Ireland during 1987 – 1988. One in every nine of the hectads of the BSBI grid (11%) was sampled systematically (Fig. 2.1.1). (The BSBI grid follows the eastings and northings of the Ordnance Survey of Great Britain (OSGB) national grid mapping system). Within each hectad, three tetrads were selected for more detailed recording. These were tetrads A, J and W following standard BSBI terminology (Fig. 2.1.2). These tetrads were selected to be as geographically separated as possible. In some cases, such as coastal areas, only two tetrad samples could be made within the selected hectad. Volunteer recorders were asked to concentrate recording effort within these tetrads and to make three separate visits to provide detailed data. For a complete description of the BSBI Monitoring Scheme see Rich and Woodruff (1990, 1996).

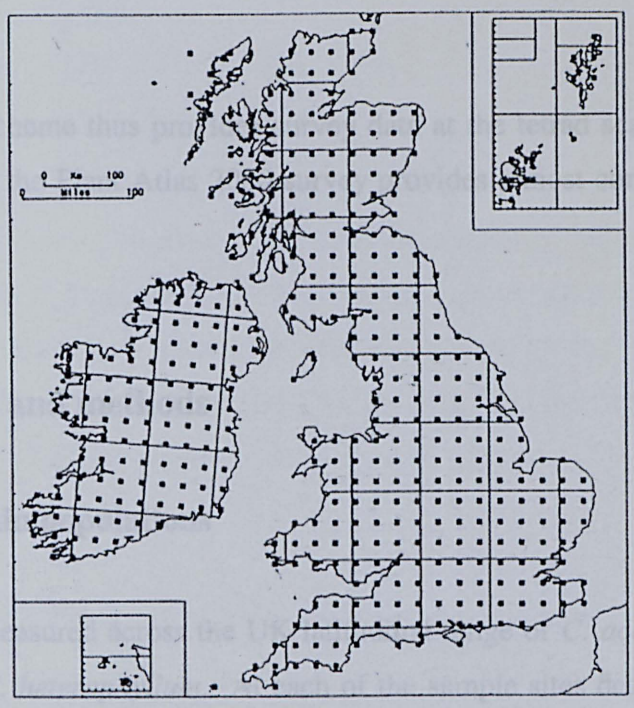

Fig. 2.1.1 Hectads selected for the BSBI Monitoring Scheme. (Reproduced from Rich and Woodruff (1990))

E	J	P	U	Z
D	I	N	T	Y
C	H	M	S	X
B	G	L	R	W
A	F	K	Q	V

Fig. 2.1.2 Nomenclature of tetrad survey areas lying within a hectad. Tetrads surveyed for the BSBI Monitoring Scheme are highlighted in red

The Plant Atlas 2000 project was a hectad survey of Britain and Ireland. This was conducted over the period 1987 – 2001 recording plant species present in every hectad of the BSBI grid.

The Monitoring Scheme thus provides survey data at the tetrad scale but with limited coverage, whereas the Plant Atlas 2000 survey provides almost complete coverage but at the hectad scale.

2.2.1 Materials and methods

Abundance within populations

Abundance was measured across the UK latitudinal range of *C. acaule*, *C. arvense*, *C. eriophorum* and *C. heterophyllum*. At each of the sample sites detailed in Table 1.3.1 and represented in Fig. 1.3.1 abundance was measured as the maximum density of individuals within a population.

Maximum density was calculated based on samples of randomly placed quadrats in the densest area of the population. Population size was sometimes constrained by the area of available habitat (an anthropogenic effect) whilst in other cases populations occurred in an area of a larger patch of apparently suitable habitat. Density of individuals in the constrained populations appeared to drop much more rapidly toward the population margin. Abundance was not assessed based on the entire population area as this edge effect differed between these groups.

For *C. arvense* and *C. heterophyllum* abundance was recorded as the number of shoots within two 1m × 1m quadrats at each site, for *C. acaule* and *C. eriophorum* abundance was recorded from two 5m × 5m quadrats. In *C. acaule* abundance was based on the number of clumps rather than individual shoots. Quadrat size was larger for *C. acaule* and *C. eriophorum* since these species occur as scattered individuals rather than the dense stands that are typical of *C. arvense* and *C. heterophyllum*. For each species mean abundance per site was regressed against latitude. Non-linear regression and

calculation of regression confidence intervals was performed using SigmaPlot 2001 for Windows v7.0 (SPSS inc. Chicago, IL, USA). Patterns of population abundance with latitude are shown in Fig. 2.3.1 – 2.3.4.

Population frequency

This analysis used distributional data for *Cirsium acaule*, *C. arvense*, *C. eriophorum* and *C. heterophyllum* from the BSBI Monitoring Scheme and the Plant Atlas 2000 project. Permission to use these data sets was given by the Department for the Environment, Transport and Regions (DETR), the BSBI and the Centre for Ecology and Hydrology (CEH). The data were provided by Chris Preston of CEH, (Monks Wood, Abbots Ripton, UK).

If we assume the Plant Atlas 2000 species survey to be the most reliable indicator of a species geographic range within mainland UK, then the Monitoring Scheme tetrad species survey can be compared with this to provide a more accurate surrogate for population frequency data. For any tetrad sample in the monitoring scheme we know from the Plant Atlas 2000 survey whether the species of interest occurs within the hectad from which the tetrad sample is taken. The more frequently populations occur within that hectad, the greater the likelihood that they will be present in a tetrad sub-sample. Thus a decline in population frequency will be represented by a decline in the number of tetrads per hectad that include the species of interest.

Data were compared using a FORTRAN 77 programme written by M.R. Lomas of the University of Sheffield, Department of Animal and Plant Sciences. The Plant Atlas 2000 hectad survey provided the baseline record against which the Monitoring Scheme data were compared.

A frequency score was calculated by dividing the number of tetrad samples containing the species of interest within any sampled 10 km square by the number of tetrad samples taken. Frequency scores were 3/3 2/3 1/2 1/3 or 0 (where 3/3 represents presence in each of three samples taken, 1/2 represents presence in one sample out of two taken and 0 represents absence in all samples). As tetrad samples have been taken

only within hectads where the species of interest is known to occur, a score of 0 indicates lowest frequency within this hectad rather than absence. To reflect this, frequency scores were expressed on a scale ranging from 5 (most frequent) – equivalent to a frequency score of 3/3, to 1 – equivalent to a frequency score of 0. Maps showing population frequency of the four *Cirsium* species throughout mainland UK are shown in Fig. 2.3.5.

The northings of the OSGB national grid do not correspond perfectly with lines of latitude, therefore mean latitude was calculated for each northing position at which surveys were made. For each species the arithmetic mean of population frequency was then calculated for all hectad samples at each sampling latitude. Maximum and mean frequency were then regressed against latitude. Non-linear regression and calculation of regression confidence intervals was performed using SigmaPlot 2001 for Windows v7. Mean and maximum frequency of *Cirsium* sp. with latitude is shown in Fig. 2.3.6 – 2.3.9.

2.3.1 Results

Abundance within populations

There was a significant relationship between latitude and within population abundance only in *C. heterophyllum* (R^2 = 0.37, $P < 0.05$, Fig. 2.3.4). Abundance in *C. heterophyllum* is highest in the core area of its UK distribution in central Scotland and declines approaching its southern range edge. No relationship was found between latitude (the proxy for proximity to range edge) and abundance in any other species (Fig. 2.3.1– 2.3.3, summary data presented in Appendix C).

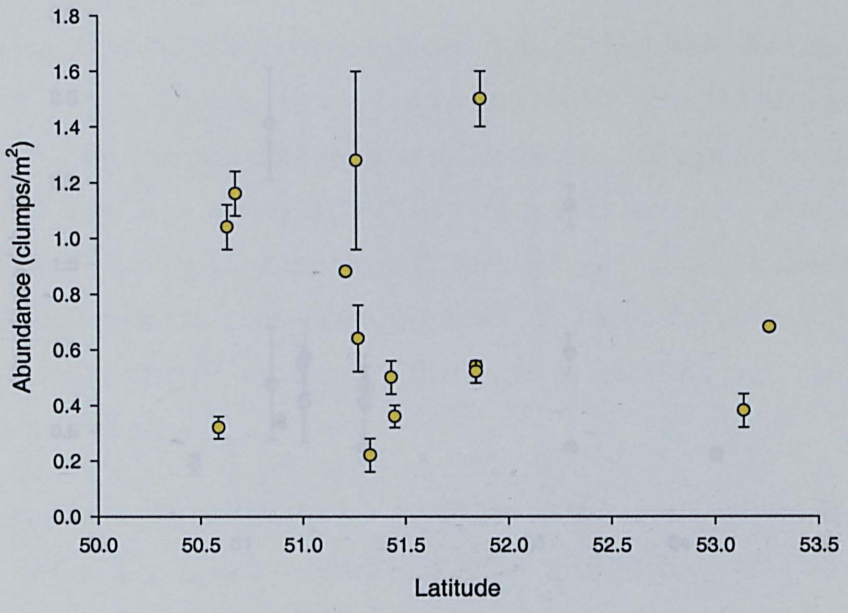

Fig. 2.3.1 Mean abundance (± 1 SE) of *Cirsium acaule* as a function of latitude

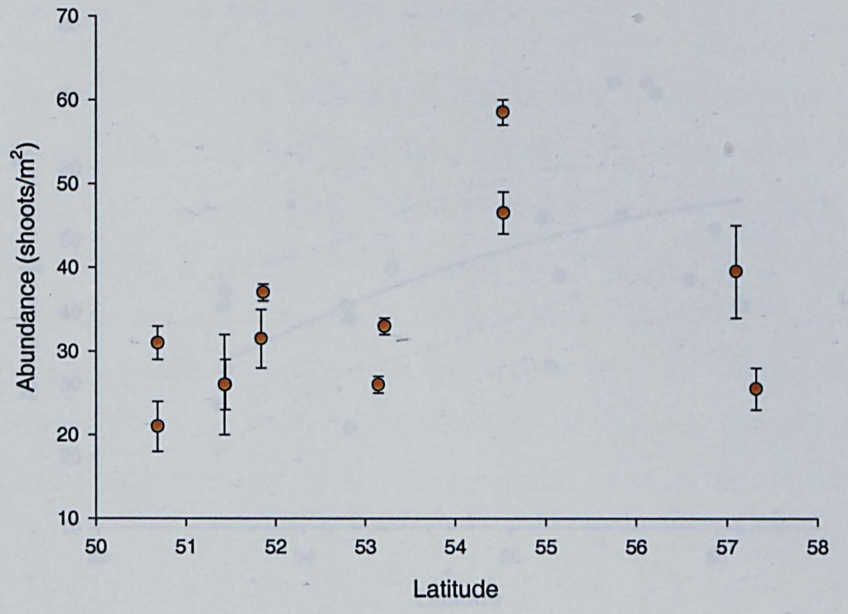

Fig. 2.3.2 Mean abundance (± 1 SE) of *Cirsium arvense* as a function of latitude

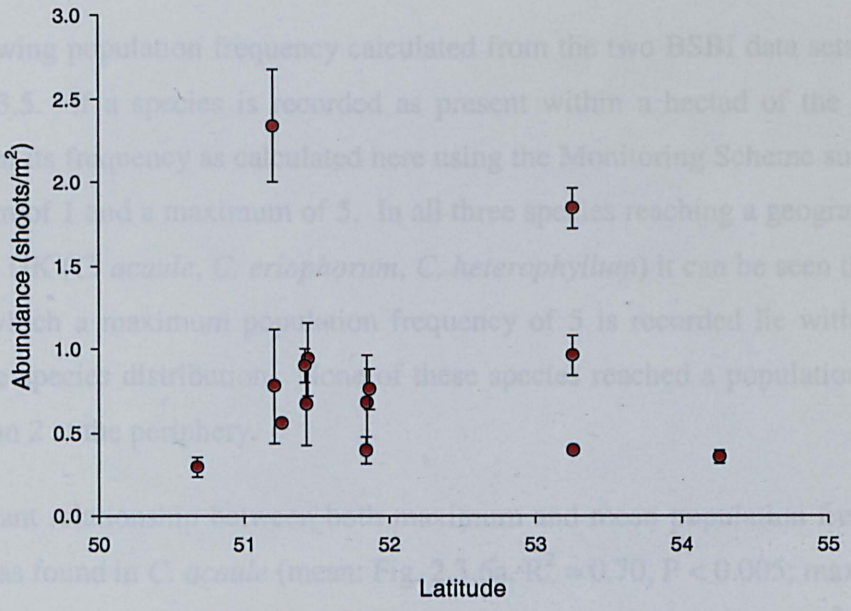

Fig. 2.3.3 Mean abundance (± 1 SE) of *Cirsium eriophorum* as a function of latitude

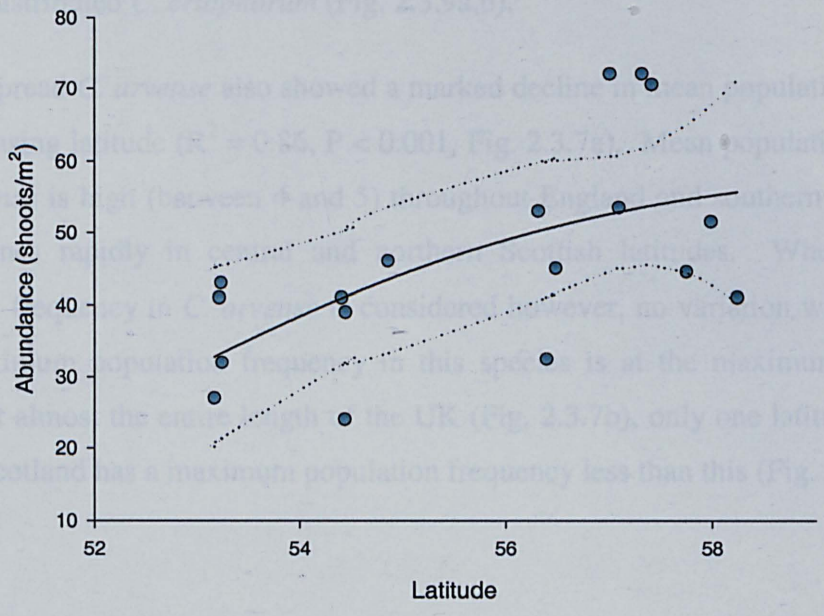

Fig. 2.3.4 Mean abundance of *Cirsium heterophyllum* as a function of latitude. Regression: $y = -1830 + 62.9x - 0.524x^2$, $R^2 = 0.37$, $P < 0.05$. Dotted lines show 95% confidence interval of regression

Population frequency

Maps showing population frequency calculated from the two BSBI data sets are shown in Fig. 2.3.5. If a species is recorded as present within a hectad of the Atlas 2000 survey then its frequency as calculated here using the Monitoring Scheme survey can be a minimum of 1 and a maximum of 5. In all three species reaching a geographical limit within the UK (*C. acaule*, *C. eriophorum*, *C. heterophyllum*) it can be seen that the only areas in which a maximum population frequency of 5 is recorded lie within the core area of the species distribution. None of these species reached a population frequency greater than 2 at the periphery.

A significant relationship between both maximum and mean population frequency and latitude was found in *C. acaule* (mean: Fig. 2.3.6a, $R^2 = 0.70$, $P < 0.005$; maximum: Fig. 2.3.6b, $R^2 = 0.82$, $P < 0.001$) and *C. heterophyllum* (mean: Fig. 2.3.8a, $R^2 = 0.41$, $P < 0.05$; maximum: Fig. 2.3.8b, $R^2 = 0.42$, $P < 0.05$). Both species show a curvilinear relationship between population frequency and latitude, with high population frequency in core areas of the species range and a decrease approaching the range boundary. No significant relationship between population frequency and latitude was seen in the more narrowly distributed *C. eriophorum* (Fig. 2.3.9a,b).

The widespread *C. arvense* also showed a marked decline in mean population frequency with increasing latitude ($R^2 = 0.86$, $P < 0.001$, Fig. 2.3.7a). Mean population frequency in *C. arvense* is high (between 4 and 5) throughout England and southern Scotland but then declines rapidly in central and northern Scottish latitudes. When maximum population frequency in *C. arvense* is considered however, no variation with latitude is seen. Maximum population frequency in this species is at the maximum value of 5 throughout almost the entire length of the UK (Fig. 2.3.7b), only one latitude in the far north of Scotland has a maximum population frequency less than this (Fig. 2.3.5).

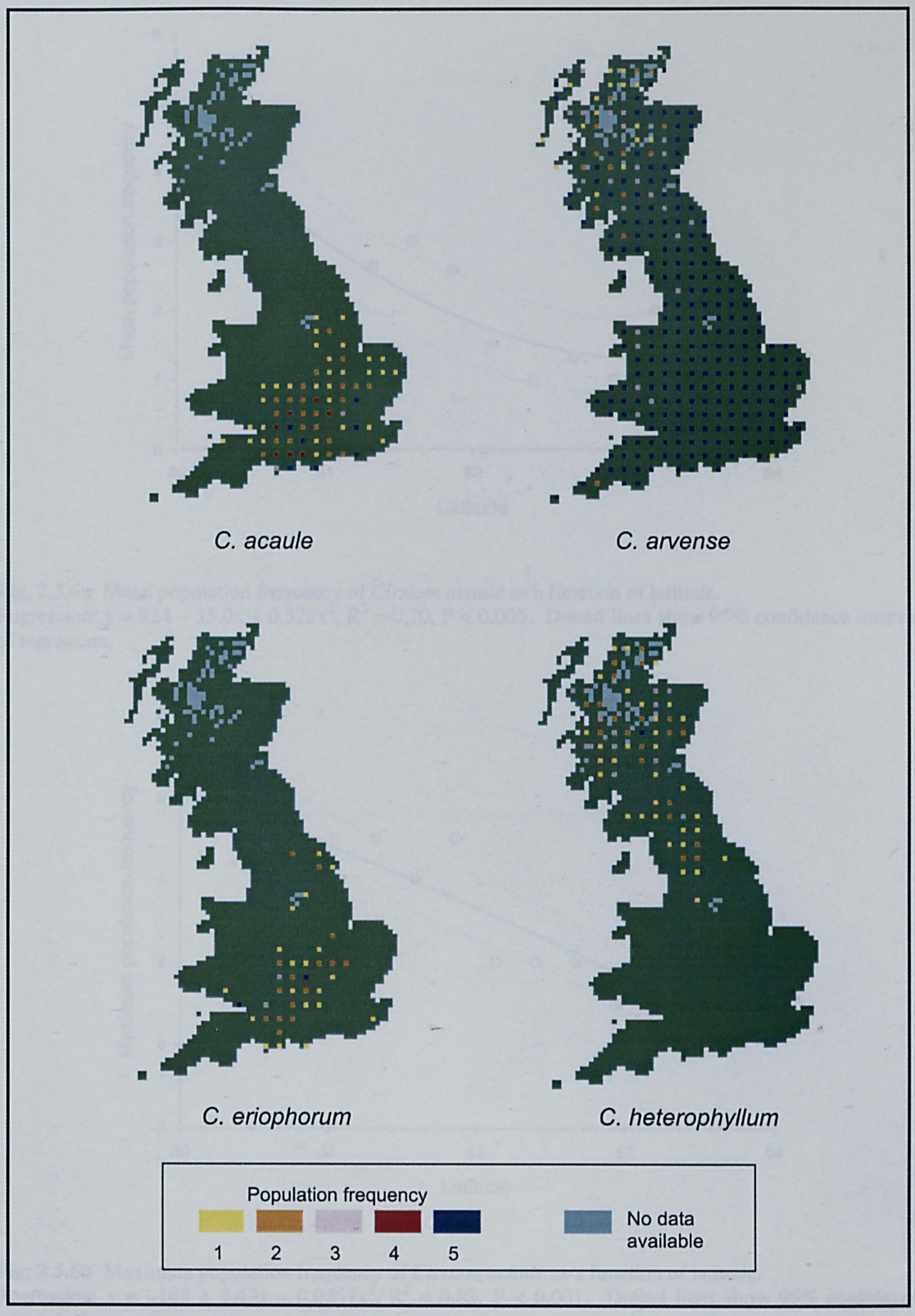

Fig. 2.3.5 Population frequency of *Cirsium* species within hectad survey areas throughout the UK; 1 represents lowest frequency

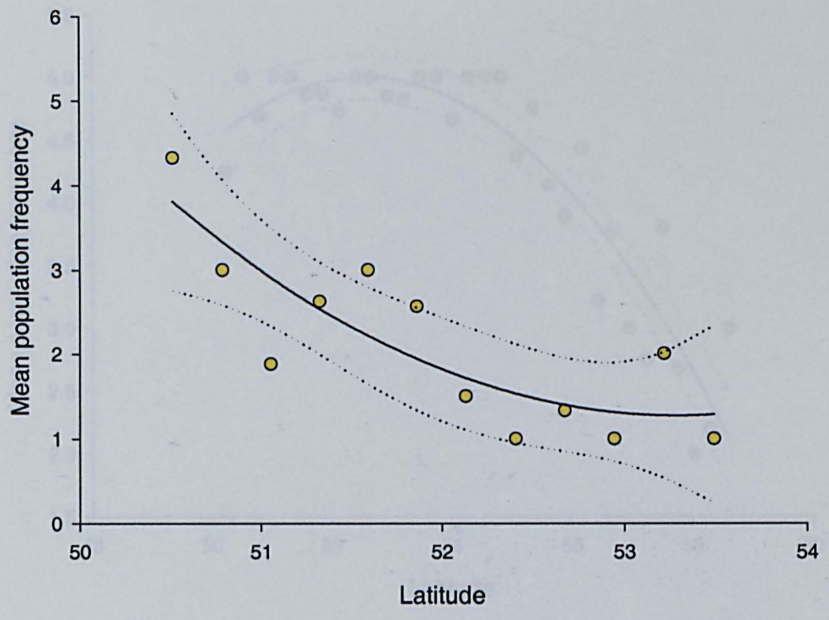

Fig. 2.3.6a Mean population frequency of *Cirsium acaule* as a function of latitude. Regression: $y = 934 - 35.0x + 0.328x^2$, $R^2 = 0.70$, $P < 0.005$. Dotted lines show 95% confidence interval of regression.

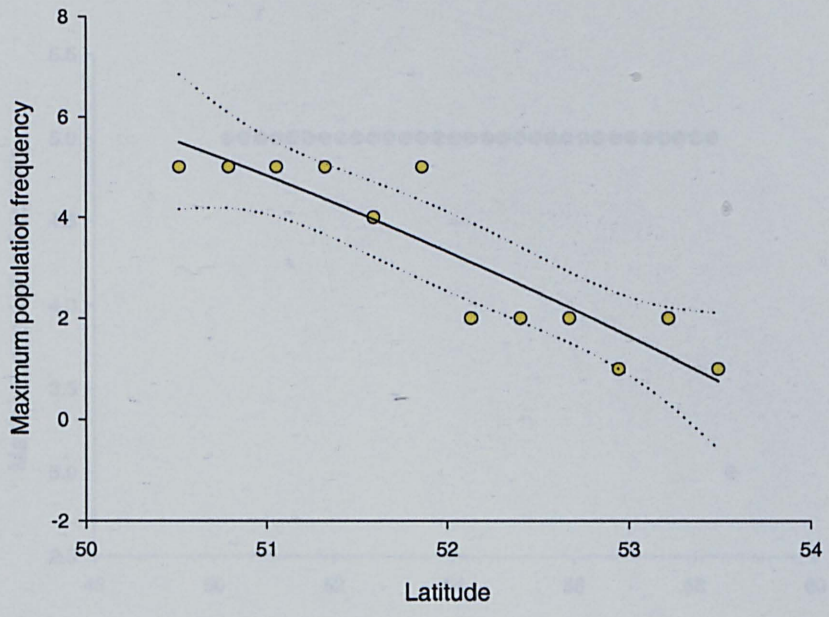

Fig. 2.3.6b Maximum population frequency of *Cirsium acaule* as a function of latitude. Regression: $y = -148 + 7.43x - 0.0867x^2$, $R^2 = 0.82$, $P < 0.001$. Dotted lines show 95% confidence interval of regression

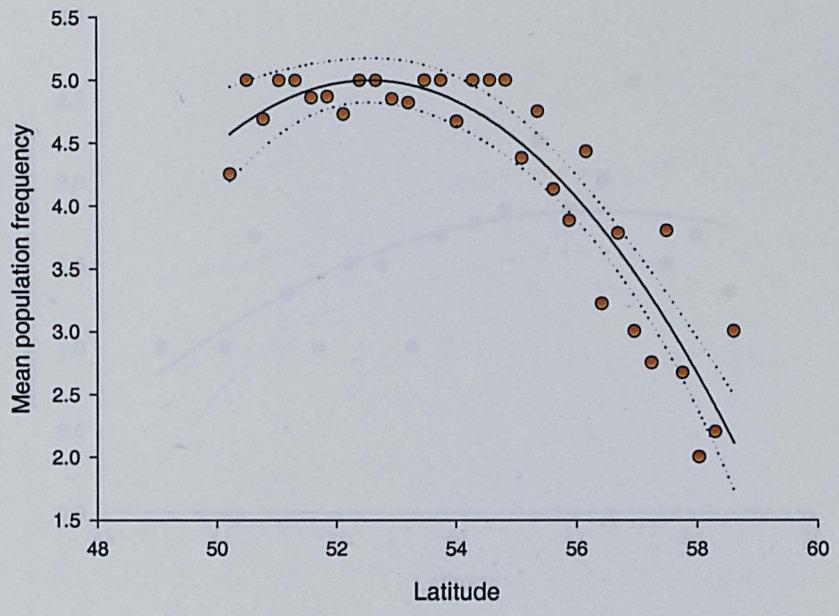

Fig. 2.3.7a Mean population frequency of *Cirsium arvense* as a function of latitude. Regression: $y = -213 + 831x - 0.0790x^2$, $R^2 = 0.86$, $P < 0.001$. Dotted lines show 95% confidence interval of regression

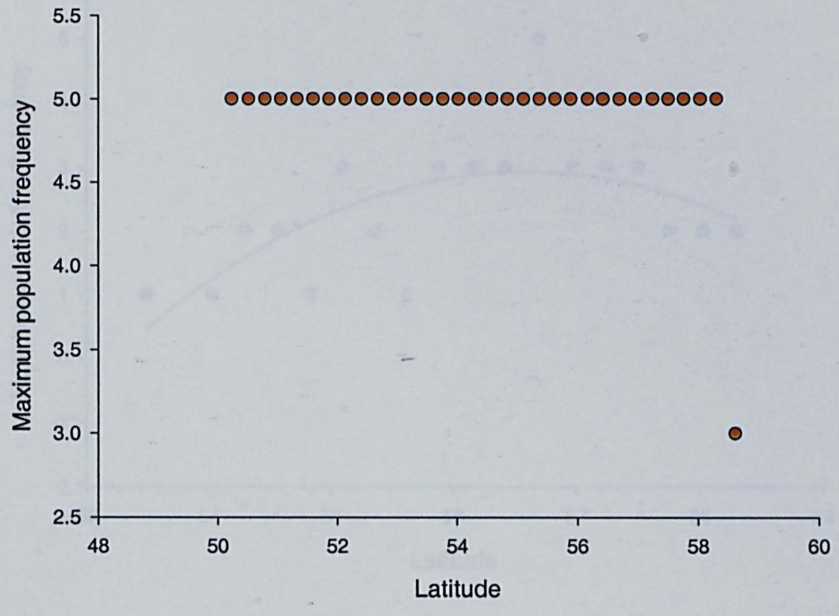

Fig. 2.3.7b Maximum population frequency of *Cirsium arvense* as a function of latitude

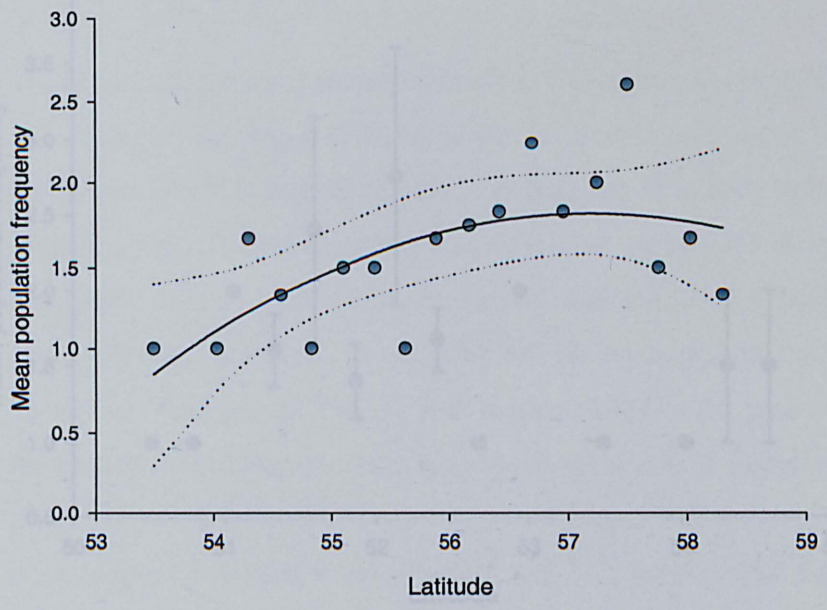

Fig. 2.3.8a Mean population frequency of *Cirsium heterophyllum* as a function of latitude. Regression: $y = -231 + 8.14x - 0.0712x^2$, $R^2 = 0.41$, $P < 0.05$. Dotted lines show 95% confidence interval of regression

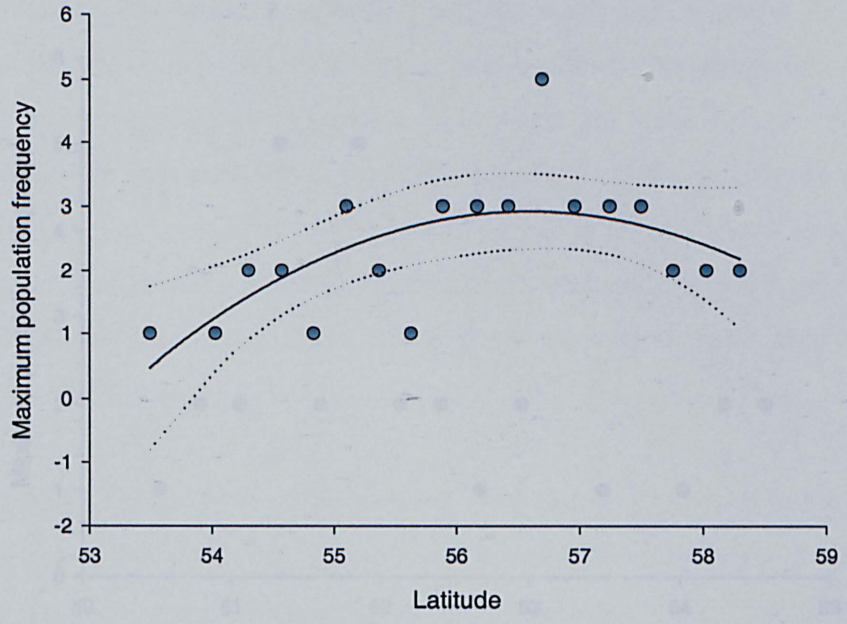

Fig. 2.3.8b Maximum population frequency of *Cirsium heterophyllum* as a function of latitude. Regression: $y = -813 + 28.8x - 0.255x^2$, $R^2 = 0.42$, $P < 0.05$. Dotted lines show 95% confidence interval of regression

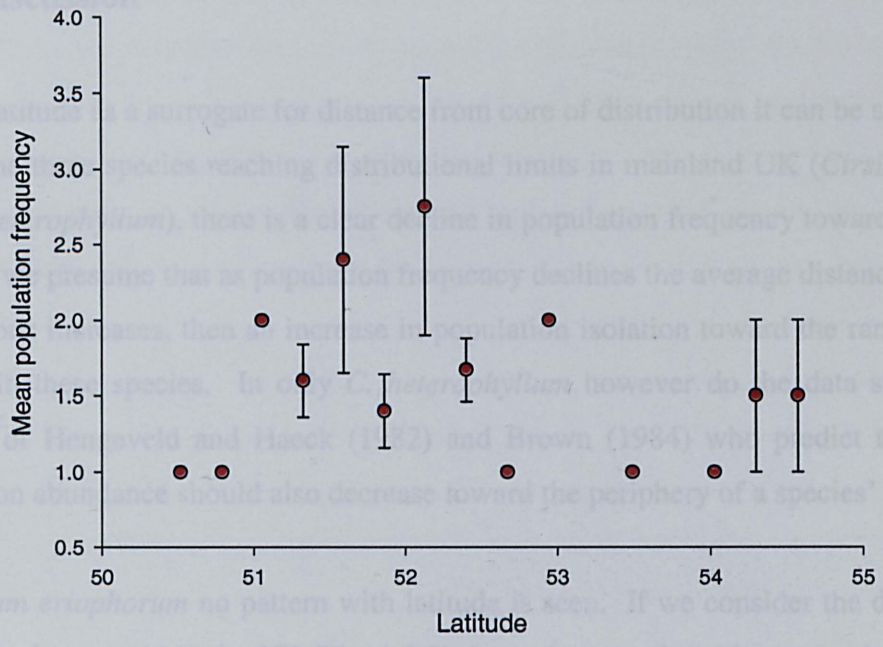

Fig. 2.3.9a Mean population frequency (± 1 SE) of *Cirsium eriophorum* as a function of latitude.

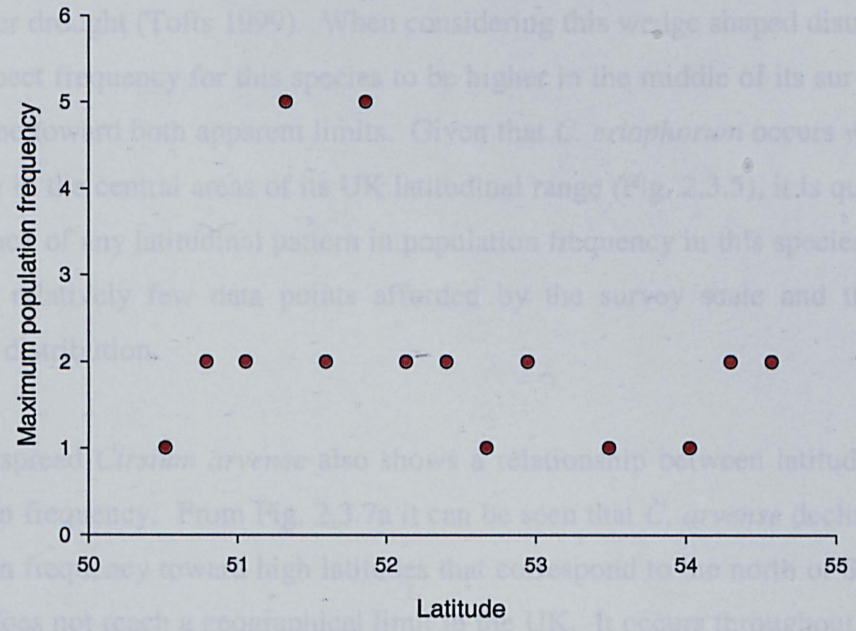

Fig. 2.3.9b Maximum population frequency of *Cirsium eriophorum* as a function of latitude.

2.4.1 Discussion

Taking latitude as a surrogate for distance from core of distribution it can be seen that in two of the three species reaching distributional limits in mainland UK (*Cirsium acaule* and *C. heterophyllum*), there is a clear decline in population frequency toward the range edge. If we presume that as population frequency declines the average distance between populations increases, then an increase in population isolation toward the range edge is evident in these species. In only *C. heterophyllum* however do the data support the theories of Hengeveld and Haeck (1982) and Brown (1984) who predict that within population abundance should also decrease toward the periphery of a species' range.

In *Cirsium eriophorum* no pattern with latitude is seen. If we consider the distribution of *C. eriophorum* within the UK (Fig. 1.2.3), it can be seen that this species has a wedge shaped distribution, running from Dorset in the south-west of the UK to Northumbria and East Anglia in the east. The UK distribution of *C. eriophorum* appears to include two distributional boundaries. It is absent from both the north-west of the UK, where it may be limited by low winter temperature and the south-east, where it may be limited by summer drought (Tofts 1999). When considering this wedge shaped distribution, we might expect frequency for this species to be higher in the middle of its surveyed range and decline toward both apparent limits. Given that *C. eriophorum* occurs with greatest frequency in the central areas of its UK latitudinal range (Fig. 2.3.5), it is quite possible that the lack of any latitudinal pattern in population frequency in this species may result from the relatively few data points afforded by the survey scale and this species' restricted distribution.

The widespread *Cirsium arvense* also shows a relationship between latitude and mean population frequency. From Fig. 2.3.7a it can be seen that *C. arvense* declines in mean population frequency toward high latitudes that correspond to the north of Scotland. *C. arvense* does not reach a geographical limit in the UK. It occurs throughout Europe and is absent only from Svalbard in the extreme north (Tutin *et al.* 1976). Fig. 1.2.2 shows the distribution of *C. arvense* throughout the UK. When compared with Fig. 2.4.1 (showing high altitude regions of the UK) it can be seen that *C. arvense* is absent from many hectads containing land over 2,500ft (762m) above sea level. Preston *et al.*

(2002) list *C. arvense* as mainly occurring from sea level to 700m with an extreme of 845m. When we consider maximum population frequency (Fig. 2.3.7b), there is no decline with latitude in *C. arvense* unlike the other *Cirsium* species investigated here, all of which display parallel patterns in mean and maximum frequency. It is likely that the apparent decline in population frequency with latitude in the UK is an altitudinal, rather than latitudinal effect, resulting from the high incidence of high altitude land in central and northern Scotland.

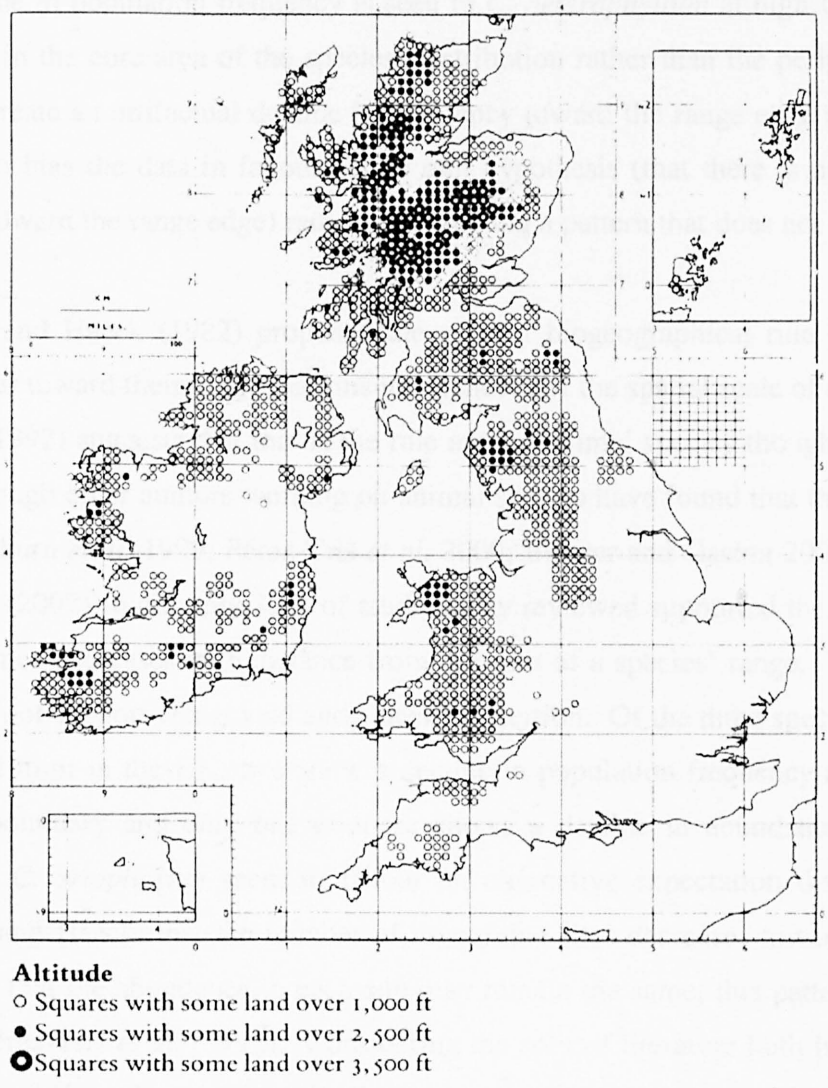

Fig. 2.4.1 High altitude areas of the UK. Reproduced from Perring and Walters (1990)

Given that *Cirsium arvense* may display a decline in local frequency across higher altitude areas of Scotland, we might expect *C. heterophyllum* to show a similar decline over the same region of its distribution. If *C. heterophyllum* also shows variation in population frequency linked to altitude in some areas of its range then potentially this could confound investigation into latitudinal patterns across the geographic range. Like *C. arvense*, *C. heterophyllum* does not reach a northern latitudinal limit in the UK (Tutin *et al.* 1976), in the UK *C. heterophyllum* commonly occurs up to 760m with a possible maximum of 975m (Preston *et al.* 2002). A slight decline in mean population frequency of *C. heterophyllum* is indeed seen at higher UK latitudes and this is mirrored in the decline in maximum population frequency over the same area. Even though a slight decline in population frequency is seen in *C. heterophyllum* at high UK latitudes this occurs in the core area of the species' distribution rather than the periphery. This could not create an artifactual decline in frequency toward the range edge as the effect would be to bias the data in favour of the null hypothesis (that there is no decline in frequency toward the range edge) rather than creating a pattern that does not exist.

Hengeveld and Haeck (1982) proposed the general biogeographical rule that species become rarer toward their range margins independent of the spatial scale of observation. Svensson (1992) suggests that this is the rule among animal species though not among plants, although other authors working on animal species have found that this is not the case (Blackburn *et al.* 1999; Pérez-Tris *et al.* 2000; Brewer and Gaston 2002). Sagarin and Gaines (2002) found only 39% of studies they reviewed supported the assumption that abundance decreases with distance from the core of a species' range. The data on *Cirsium* do not support Hengeveld and Haeck's assertion. Of the three species reaching a latitudinal limit in the UK, two show a decline in population frequency approaching the range boundary and only one of these shows a decline in abundance. *Cirsium acaule* and *C. eriophorum* seem to favour an alternative expectation discounted by Hengeveld and Haeck, that the number of favourable sites decreases toward the range margin, but that the abundance in each site may remain the same; this pattern was also found by Pérez-Tris *et al.* (1992). Considering the split of literature both in support of Hengeveld and Haeck's theory and in disagreement with it, it seems unlikely that it can be described as a general rule, at least not across all spatial scales.

A number of related theories have been proposed to explain the predicted decline in abundance toward the periphery of a species range, whether in terms of a decrease in favourable niche dimensions (Hutchinson 1957; Brown 1984; Brown *et al.* 1995) or an integrator of them such as energy balance (Hall *et al.* 1992). Pérez-Tris *et al.* (2000) explain the lack of an apparent pattern in the robin, *Erithacus rubecula* in terms of avoidance, rather than tolerance of somehow unsuitable habitat; whilst Brewer and Gaston (2002) note that their system violates certain assumptions of Brown's (1984) theory.

Brown argues that (a) if the abundance and distribution of species are determined by combinations of many physical and biotic variables, and that spatial variation in population density reflects the probability density distribution of the required combinations of these variables, and (b) some sets of variables are distributed independently of each other and environmental variation is spatially autocorrelated, then density should be highest at the centre of a species range.

Brown suggests that one type of exception to his rule would occur when instead of density being determined by the combined effects of many variables, a rapid environmental change causes one factor (or several covarying factors) to assume overwhelming importance. When we consider the scenario where one environmental variable plays the dominant role in determining the limits to a species distribution, Brown's rule would not hold even in the absence of a rapid environmental change if beyond a certain level of that variable the species shows a major response to a small degree of variation. Presence or absence in a site would then be dependent on a 'threshold effect' of a single variable (Brown 1984) and the species' border defined by a steep loss in suitable habitat rather than a gradual increase in environmental restrictions in the occupied sites (Lawton 1993; Blackburn *et al.* 1999). Such a model may be typical of many plant species (Pigott 1968; Pigott and Huntley 1981; Woodward 1996) and is believed to apply to both *Cirsium acaule* (Pigott 1968) and *C. eriophorum* (Tofts 1999). *Cirsium acaule* has been found to be particularly sensitive to summer irradiance. Establishment of populations at the northern UK distributional limit of *C. acaule* is confined almost wholly to calcareous slopes facing south-south-west where energy gain is maximized, whereas establishment occurs on slopes of many aspects in its core regions. A similar mechanism is believed to operate in C *eriophorum* (Tofts 1999).

Cirsium acaule and *C. eriophorum* may thus violate the first of Brown's assumptions and perhaps also the second, as sites fulfilling these energy requirements may occur throughout their range, though they are likely to decline in frequency toward the periphery. Since this pattern of response could lead to the avoidance (through failure to establish a population) rather than tolerance of less than suitable habitat, it is possible to envisage how this could lead to a pattern of distribution in these species where frequency but not abundance declines approaching the range boundary.

Blackburn *et al.* (1999) discovered little evidence to support the predicted decline in abundance approaching the range edge of British bird species. They suggest a number of sampling and analysis effects that could result in the failure to detect such a pattern although they show that none of these appears to explain the lack of an observed decline in their data. One proposed explanation is that this pattern may be apparent only across a narrow range directly adjacent to the range edge. Should this be the case in *Cirsium acaule* and *C. eriophorum* it is quite possible that the small number of sampling locations in peripheral areas could fail to detect a decline in abundance approaching the range edge of these species. An alternative pattern that could apply to these data is that of a decline in the range of abundance values from core to peripheral areas of the species range. Kiflawi *et al.* (2000) described sites in the core areas of a number of mollusc species as having high or low abundance whereas abundance in peripheral areas was uniformly low. Further sampling would be necessary to assess the possibility of these alternative patterns describing abundance in *C. acaule* and *C. eriophorum.*

The data presented here suggest that populations in the *Cirsium* species investigated do become less frequent in peripheral regions of the species distribution. Although insufficient sample size may have limited the detection of patterns in the abundance of these species, there is only a slight indication that abundance may vary in a similar way. Thus, although populations may become more isolated approaching the range boundary the abundance of individuals within the population does not necessarily decrease.

Chapter 3: Plant communities in which *Cirsium* species occur across their UK geographic range

3.1.1 Introduction

Plant communities are assemblages of species that change over space and time (Huntley 1991). As environmental conditions are expected to vary across a species' range (Brown 1984), peripheral populations of a species may be subject to environmental conditions that are different from those experienced by those in more central areas of the species' range (Safriel *et al.* 1994). Consequently it has been predicted that peripheral populations will occur in novel or atypical habitats (Lesica and Allendorf 1995) and hence unusual species assemblages. Differences in habitat between core and peripheral populations may lead to divergent natural selection and result in genetic divergence of populations at the edge of a species' range (Lesica and Allendorf 1995, Shreeve *et al.* 1996).

Hengeveld and Haeck (1982) and Brown (1984) predict that peripheral populations should be less abundant and more isolated than core populations. This stems from the observation that environmental factors frequently change in a clinal manner (Endler 1977), thus the favourability of a species' environment is presumed to decline from the core to the periphery of a species' range (Brown 1984). Even in the absence of a decline in abundance (the density of individuals within a population), a decline in environmental favourability is likely to result in a decrease in suitable habitat toward the range edge and therefore increasing isolation of geographically peripheral populations (Brown 1984; Lawton 1993).

A distinction can be made between ecologically marginal and geographically marginal (peripheral) populations. Strictly speaking, peripheral populations are separated from central populations by spatial distance, while ecologically marginal populations experience different biotic or abiotic environments (Lesica and Allendorf 1995). Whereas ecologically marginal populations may occur throughout the geographic range

of a species, peripheral populations occur at the range boundary by definition. In many cases peripheral populations are likely to be ecologically marginal as well.

Populations of species are often found in a greater variety of habitat types near the centre of their range compared to the edges (Hall *et al.* 1992; Pérez-Tris *et al.* 2000) as a result of the predicted decline in environmental favourability toward the range edge (Brown 1984). Brown's (1984) theory suggests that as environmental favourability declines with distance from the range centre, peripheral populations will be restricted to only the most favourable habitat type; at the periphery, even the species' 'typical' habitat is likely to become ecologically marginal. Lesica and Allendorf (1995) predict that conditions at the species boundary are likely to be different even if they are not less favourable with species frequently occurring in unusual or atypical habitats at the range edge.

There are numerous examples of disjunct populations of species occurring in novel or atypical habitats. For example, Pigott and Walters (1954) and Pigott (1956) report a mixing of typically arctic-alpine and continental species in the limestone pavements of North Yorkshire (UK) and 'The Burren' of County Clare (Republic of Ireland), whereas Barden (2000) reports that at its geographical periphery, populations of the common shrub, *Quercus ilicifolia* occur only in the rare 'low elevation rocky summit' plant community. Gankin and Major (1964) review a number of examples of unusual plant communities containing species which are disjunct from their typical edaphic or geographic range. These examples include both widespread and locally endemic species from a range of climate zones.

Changes in community composition can be used to indicate even small differences in habitat (Crozier and Boerner 1984; Dibble *et al.* 1999; Fuller *et al.* 2001). The occurrence of populations in atypical community types is often explained in terms of a reduction in competition with locally occurring species as a result of novel edaphic factors or disturbance regimes (Gankin and Major 1964; Barden 2000). Whether mediated through disturbance or edaphic factors the conclusion is the same: plants occurring at their distributional limits will often be found in novel community types, where the effects of interspecific competition and decreased environmental favourability are ameliorated.

3.2.1 Materials and methods

Community composition was used to indicate habitat type since any change in habitat will be reflected in alteration of the abundance or occurrence of species within the plant community. Community surveys were carried out during July and August 2000 within the populations listed in Table 1.3.1. Two surveys were made in each population (Listed as A and B following the site codes given Table 1.3.1). Percent shoot frequency of each species present was calculated based on presence in each of 25 cells of a 50 cm × 50 cm strung quadrat (Goldsmith *et al.* 1986). Quadrats were placed randomly within the most dense area of the population. Rose (1991) and Hubbard (1992) were used to aid identification. Quadrat records are listed in Appendix A.

Approximate affinities of the surveyed communities to the communities of the National Vegetation Classification (NVC) were identified using TABLEFIT (Hill 1996).

Data analysis

The principal aim of the community survey was to determine whether or not peripheral populations of the *Cirsium* species investigated occur in atypical habitats when compared with populations that occur in the core areas of the species geographic range.

Data reduction techniques such as classification and ordination are frequently used to identify phytosociological clusters of samples. In applying such methods, the aim is usually to identify core clusters of the most similar samples that define a particular phytosociological group. The aim of the survey presented here is not to identify clusters of the most similar samples within the survey but instead to identify those that are least typical of any group (ie - the samples that are most remote from the cluster cores). The approach taken was to perform an initial clustering of the data according to the similarity of samples and then to discover the samples that were most remote from the cores of any clusters identified.

A potential problem with this approach is that different methods for the analysis of community data are likely to deal with outlying samples in different ways (Gauch and Whittaker 1981). The most informative approach is thus to use several different

methods of analysis on the same data set and compare the results to get a more comprehensive view of the data structure (Kent and Ballard 1988).

Community data were analysed using both classification and ordination methods.
Two classification approaches were used reflecting the major division in classification techniques (outlined below). These were a divisive clustering approach using the programme *TWINSPAN* v4.0 (Hill 1979a), part of the VESPAN III for Windows package, (Malloch 1999) and an agglomerative clustering approach using the package *ClustanGraphics* v5 (Wishart 1999). The ordination method used was detrended correspondence analysis (DCA) using the programme *DECORANA* (Hill 1979b), modified from the original by Malloch (1999). The aims and methods of the community data analysis were discussed with the author of each programme to ensure the validity of the approach. A detailed description of these techniques can be found in Kent and Coker (1992)

Outline of classification and ordination methods

The principal division between the approaches used in TWINSPAN and ClustanGraphics is that of divisive versus agglomerative clustering techniques. Divisive clustering begins with a single cluster containing all samples and splits the cluster into two sub-groups based on the major divisions in the data set. Each sub group is then divided in the same way and the sub-groups produced divided again etc. etc. until a specified number of groups is obtained. Agglomerative clustering begins with all samples as individual clusters and progressively combines them in terms of their similarity until all of the samples are in one cluster.

The divisive approach has the theoretical advantage that all of the available information in the data set is used to make the critical topmost decisions. The divisive approach therefore takes an overall view of the data whereas the agglomerative approach emphasises the details of individual sample comparisons. Where samples form natural clusters in dissimilarity space, the agglomerative technique is likely to recognise such clusters whereas the divisive technique may bisect them. As a consequence, outliers are likely to be dealt with in different ways by the two techniques – either being forced into

the closest group, or being removed as a sub group at an early stage in the analysis (Gauch and Whittaker 1981).

Given the array of techniques and programmes available for classification, TWINSPAN represents the most widely used programme employing the divisive approach and has attracted relatively little critical comment (Gauch and Whittaker 1981; Kent and Ballard 1988). ClustanGraphics allows the use of a number of different agglomerative clustering methods together with methods for the identification of samples that are remote from or intermediate between the cores of the clusters identified (Wishart 1999, 2000).

DCA is a widely used ordination method that overcomes many of the problems inherent to many of the earlier ordination methods. Despite this, it is acknowledged to have a number of drawbacks, one of which is its difficulty in dealing with outliers (Kent and Coker 1992). Based on a review of the problems of DCA and comparable methods, Kent and Coker conclude that for the analysis of ecological data 'the method is still as good as any other in most situations and better than most in many', however, like Kent and Ballard (1988) they suggest adopting several parallel approaches to community data analysis.

Method of analysis and programme options

Quadrat data were entered using the *Record* routine of VESPAN III giving the maximum quantitative value for species as 25 (the number of cells in each quadrat). The *Select* routine was then used to prepare the data for input into TWINSPAN, and DECORANA, and a FORTRAN 77 programme written by M.R. Lomas of the University of Sheffield was used to prepare the data for entry into ClustanGraphics.

TWINSPAN

TWINSPAN was run using the default analysis parameters (Malloch 1999) with the exception that pseudospecies cut levels (species frequency categories) were set at 0.10 5.10 10.10 15.10 20.10 since maximum frequency was 25. The community classification produced is presented in fig. 3.1.1.

ClustanGraphics

Stage 1. Clustering of proximities and outlier analysis:

Initial clustering of the data was performed using the increase sum of squares method. Proximities were then calculated based on squared Euclidean distance - the most commonly used measure (Kent and Coker 1992) and clustered using increase in sum of squares. This method is suggested as producing clusters that are relatively homogeneous with respect to all of the clustered variables (Wishart 1999) All samples were given equal weighting. Samples were then serialised (optimally re-ordered). Fig. 3.1.1 shows the resulting classification dendrogram.

Stage 2. FocalPoint clustering: identifying outliers and intermediates:

FocalPoint clustering allows the identification of an optimised cluster model resulting from a series of random clustering trials based on various different starting strategies. It aims to avoid problems that can arise as a result of the sensitivity of clustering procedures to sample order and allows the identification of outlying samples and those that are intermediate between clusters. The goodness of fit and reproducibility of the solution is also calculated (Wishart 2000).

Clusters were centred on exemplars rather than means since the cluster centre is then a real sample. A four cluster model was specified as this reflected the 4 *Cirsium* species split of the community survey.

Following the initial clustering, 30000 random trials were performed, split evenly between the following starting strategies. These strategies test the sensitivity to sample order of the optimum solutions found and allow measurement of their reproducibility:

1) Randomise clusters - which assigns samples randomly between the specified number of clusters before reassignment in FocalPoint analysis.

2) Tree partition - which uses the cluster means at the specified cluster level of the initial data clustering (here 4 clusters) as the starting point for the FocalPoint clustering.

3) Cluster exemplars – which uses the data points corresponding to the cluster exemplars at the specified cluster level of the initial data clustering as the starting cluster means in the FocalPoint analysis.

Outlier and intermediate analysis compares the distance of individual samples to cluster means thus it was necessary to run the analysis based on a cluster around means strategy. As the initial FocalPoint analysis had been centred on cluster exemplars, a repeat analysis was performed as above using cluster means as the cluster centres to check comparability. The resulting cluster solution was identical to that resulting from clustering around exemplars.

Outlier analysis: The analysis was repeated as above, clustering around means. A threshold of 85% of the survey data was set to identify outliers, thus the 15% of the data that were most distant from cluster cores were identified (Table 3.1.1).

Intermediate analysis: The analysis was repeated above setting the exclusion distance to 85%. This identifies a sample as an intermediate if its distance from its nearest cluster is greater than 85% of its distance from its second nearest cluster (Table 3.1.2).

DECORANA

DECORANA was run using the default analysis parameters (Malloch 1999). Ordination plots of axis 1 against axis 2 and axis 1 against axis 3 are presented in fig.3.2.1. The axes in the DCA plots represent abstract axes of floristic diversity, which can be correlated with measured environmental variables hypothesised to influence the species' composition of the communities sampled. The most remote samples of each species in the DECORANA ordination were identified by taking the six highest and lowest scores for each species on each of the first three axes of the ordination. This allowed identification of the 12 least typical samples for each species (Table 3.2.1).

3.3.1 Results

Community types

Cirsium acaule occurred mainly in species rich, short sward calcicolous grassland communities of the *Festuca ovina-Avenula pratensis* type, principally the *Holcus lanatus-Trifolium repens* sub community (CG 2c) and the typical community (CG 2)

and also *Brachypodium pinnatum*: *Avenula pratensis-Thymus praecox* (CG 4a) sub community.

The *C. arvense* stands surveyed fall into a more diverse range of communities. There is a split between sites more typical of a group of roadside and wasteland communities of an *Urtica dioica-Cirsium arvense* type (OV 25,25a,25b) (these were provisional categories not included in the NVC when TABLEFIT was written) and a range of mesotrophic grassland communities of the *Arrhenatherum elatius* (MG 1,1a,1b,1d), *Lolium perenne-Cynosurus cristatus* (MG 6,6c) and *Festuca rubra-Agrostis stolonifera-Potentilla anserina* (MG 11a) types.

The majority of *C. eriophorum* sites surveyed were categorised as *Arrhenatherum elatius* mesotrophic grasslands, largely the typical community (MG1) and the *Festuca rubra* sub community (MG 1a) and to a lesser extent within the *Cynosurus cristatus-Centaurea nigra* (MG 5a,5b) and *Lolium perenne-Cynosurus cristatus* (MG 6,6a,6b) types.

Despite the wayside situation of most of the *C. heterophyllum* survey sites, this species was classified as occurring typically in the *Festuca rubra-Holcus lanatus* maritime grassland community (MC 9) and a range of mesotrophic grassland communities of the *Arrhenatherum elatius* (MG 1,1a) and *Holcus lanatus-Deschampsia cespitosa* (MG 9,9b) types.

Sample classification

Fig. 3.1.1 shows dendrograms representing the community classifications produced by the divisive method (TWINSPAN) and the agglomerative method (ClustanGraphics).

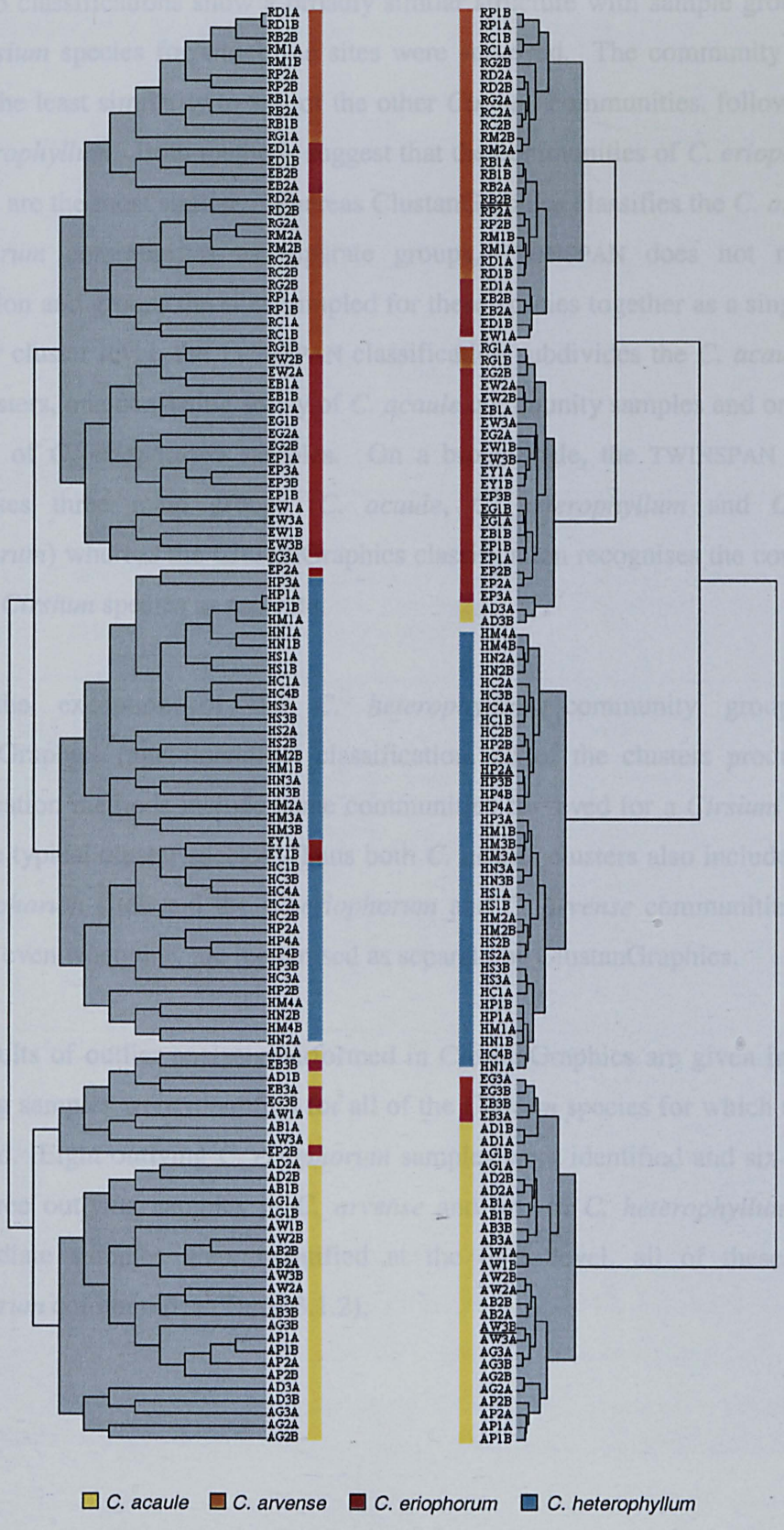

Fig. 3.1.1 Communities classified according to Twinspan (left) and ClustanGraphics (right). Coloured bars indicate the *Cirsium* species for which the site was surveyed. The most typical member of each Clustan group is underlined. Dendrograms are shaded at the four-group level. Codes for peripheral populations begin AP, EP, EY and HP. For explanation of site codes see Fig. 1.3.1and Table 1.3.1.

The two classifications show a broadly similar structure with sample groups reflecting the *Cirsium* species for which the sites were surveyed. The community of *C. acaule* shows the least similarity to any of the other *Cirsium* communities, followed by that of *C. heterophyllum.* Both methods suggest that the communities of *C. eriophorum* and *C. arvense* are the most similar. Whereas ClustanGraphics classifies the *C. arvense* and *C. eriophorum* communities as separate groups, TWINSPAN does not make such a distinction and groups the sites sampled for these species together as a single group. At the four cluster level, the TWINSPAN classification subdivides the *C. acaule* group into two clusters, one consisting solely of *C. acaule* community samples and one including a number of *C. eriophorum* samples. On a broad scale, the TWINSPAN classification recognises three main groups (*C. acaule*, *C. heterophyllum* and *C. arvense*/*C. eriophorum*) whereas the ClustanGraphics classification recognises the community type of each *Cirsium* species as separate.

With the exception of the *C. heterophyllum* community grouping in the ClustanGraphics (agglomerative) classification, all of the clusters produced by both classification methods include some communities surveyed for a *Cirsium* species other than the typical cluster species. Thus both *C. acaule* clusters also include a number of *C. eriophorum* sites and the *C. eriophorum* and *C. arvense* communities show some overlap even when they are recognised as separate by ClustanGraphics.

The results of outlier analysis performed in ClustanGraphics are given in Table 3.1.1. Outlying samples were identified for all of the *Cirsium* species for which the sites were surveyed. Eight outlying *C. eriophorum* samples were identified and six in *C. acaule*, with three outlying samples in *C. arvense* and one in *C. heterophyllum.* Only four intermediate samples were identified at the 85% level, all of these were in *C. eriophorum* communities (Table 3.1.2).

Table 3.1.1 Outlying samples as identified by FocalPoint clustering. Samples are classified as outliers if they fall outside 85% of the maximum sample distance from cluster cores. Cluster model is based on 30,000 trials

	C. acaule	*C. arvense*	*C. eriophorum*	*C. heterophyllum*
Outlying samples	AD1B, AD1A, AB1A, AW1A, AW1B, AG2A	RP1B, RP1A, RG2B	ED1A, ED1B, EW3A, EG2A, EP3A, EG3B, EB3B, EB3A	HM4A
	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Cluster members	*C. arvense* sites. Except: RG1B, RP1B, RP1A, RG2B	*C. eriophorum* sites. Except: ED1A, ED1B, EW3A, EG2A, EP3A, EG3B, EB3B, EB3A Additionally: RG1B, HC2A	*C. heterophyllum* sites. Except: HC2A, HM4A	*C. acaule* sites. Except: AD1B, AD1A, AB1A, AW1A, AW1B, AG2A
Reproducibility of cluster solution: 95%				

Table 3.1.2 Intermediate samples as identified by FocalPoint clustering. Samples are classified as intermediate if their distance from the nearest cluster is greater than 85% of their distance from the second nearest cluster. Cluster model is based on 30,000 trials

	C. acaule	*C. arvense*	*C. eriophorum*	*C. heterophyllum*
Intermediate samples	none	none	ED1A, ED1B, EP2B, EB3A	none
	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Cluster members	*C. arvense* sites Except: RG1B	*C. eriophorum* sites Except: EG3B, EB3B, ED1A, ED1B, EP2B, EB3A Additionally: RG1B, HC2A	*C. heterophyllum* sites Except: HC2A	*C. acaule* sites Additionally: EG3B, EB3B
Reproducibility of cluster solution: 97%				

Sample ordination

This slight overlap in community composition is also reflected in the sample ordination (Fig. 3.2.1.). Here as in the community classification the *C. acaule* sites form the most distinct group followed by the sites surveyed for *C. heterophyllum.* Most of the separation of the community types is accounted for by axis 1 (eigenvalue 0.66), followed by axis 2 (eigenvalue 0.40). There is little separation of any community type on the third axis of the ordination (eigenvalue 0.29) and little separation of the community types of *C. arvense* and *C. eriophorum* on any axis. For each species the samples occupying the most extreme position on the axes shown in Fig. 3.2.1 are listed in Table 3.2.1

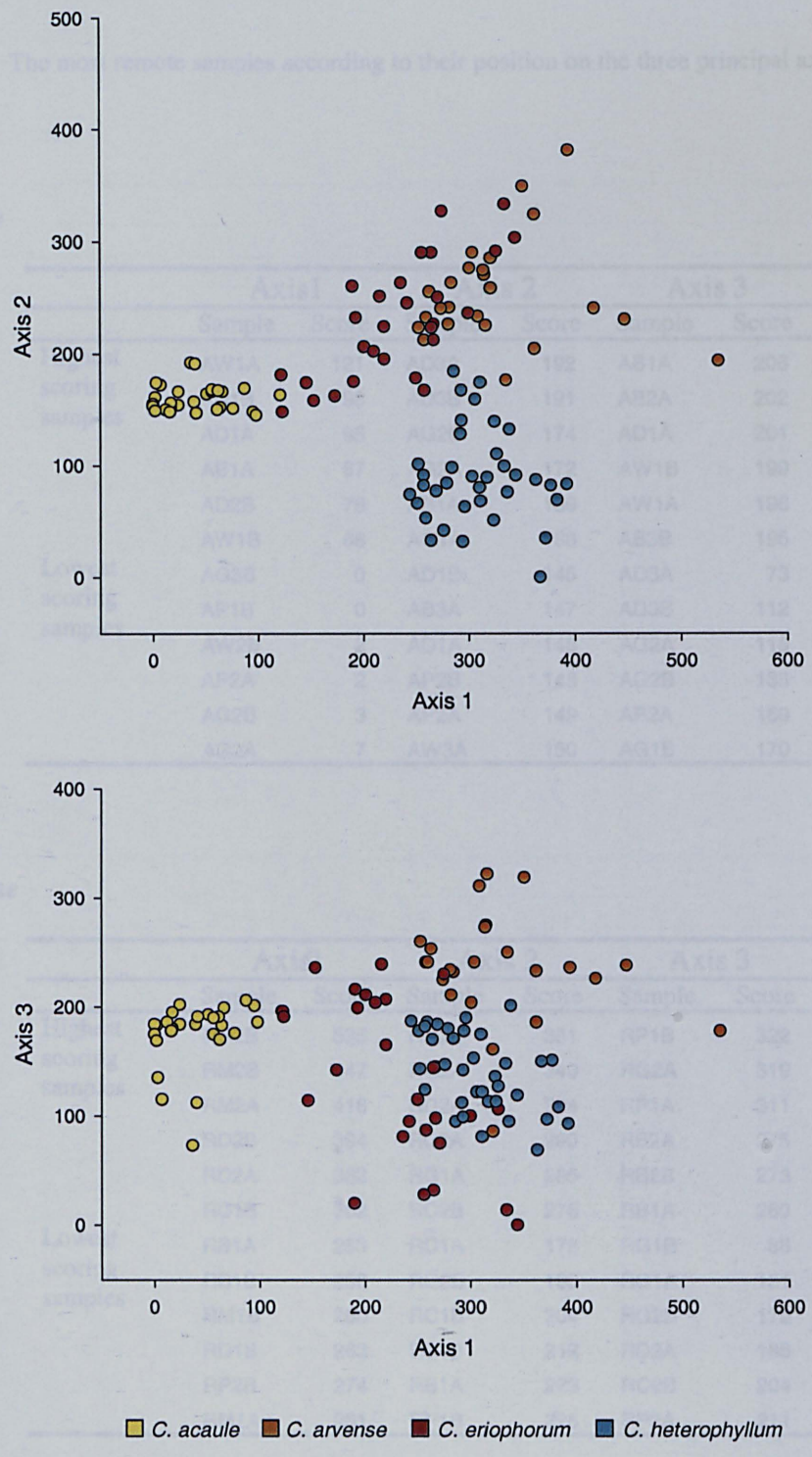

Fig. 3.2.1 Community ordination plots. axis 1 v axis 2 (top), axis 1 v axis 3 (bottom). Axes represent abstract axes of floristic diversity. Symbol colour indicates the *Cirsium* species for which the site was surveyed. Eigenvalues: axis 1, 0.66; axis 2, 0.40; axis 3, 0.29. Eigenvalues represent the relative contribution of the axis in explaining the total variation in the data.

Table 3.2.1. The most remote samples according to their position on the three principal axes of the DCA ordination.

(a) ***C. acaule***

	Axis1		Axis 2		Axis 3	
	Sample	Score	Sample	Score	Sample	Score
Highest scoring samples	AW1A	121	AD3A	192	AB1A	206
	AD1B	98	AD3B	191	AB2A	202
	AD1A	95	AG2B	174	AD1A	201
	AB1A	87	AG2A	172	AW1B	199
	AD2B	76	AB1A	169	AW1A	196
	AW1B	66	AG1A	168	AB3B	195
Lowest scoring samples	AG3B	0	AD1B	145	AD3A	73
	AP1B	0	AB3A	147	AD3B	112
	AW2B	2	AD1A	148	AG2A	115
	AP2A	2	AP2B	148	AG2B	135
	AG2B	3	AP2A	149	AP2A	169
	AG2A	7	AW3A	150	AG1B	170

(b) ***C. arvense***

	Axis1		Axis 2		Axis 3	
	Sample	Score	Sample	Score	Sample	Score
Highest scoring samples	RG2B	535	RD2B	381	RP1B	322
	RM2B	447	RG2A	349	RG2A	319
	RM2A	418	RD2A	324	RP1A	311
	RD2B	394	RC2A	290	RB2A	275
	RD2A	362	RG1A	285	RB2B	273
	RC1B	362	RC2B	276	RB1A	260
Lowest scoring samples	RB1A	253	RC1A	176	RG1B	86
	RB1B	258	RG2B	193	RG1A	161
	RM1B	260	RC1B	204	RG2B	178
	RD1B	263	RB1B	212	RD2A	186
	RP2B	274	RB1A	223	RC2B	204
	RM1A	281	RP1B	225	RP2A	211

Table 3.2.1. continued.

(c) *C. eriophorum*

	Axis1		Axis 2		Axis 3	
	Sample	Score	Sample	Score	Sample	Score
Highest scoring samples	EG2A	344	EG2B	333	ED1B	239
	EG2B	334	EW3A	327	EG3B	236
	EW3B	326	EG2A	303	EW3A	230
	EB1A	300	EW3B	291	EG3A	216
	EW3A	275	EP1A	290	ED1A	211
	EG1B	271	EP1B	290	EB2A	207
Lowest scoring samples	EB3A	122	EB3B	148	EG2A	0
	EB3B	123	EG3B	158	EG2B	14
	EP2B	146	EP2A	162	EP3A	20
	EG3B	153	EY1A	167	EP1B	28
	EP2A	173	EP2B	174	EP1A	32
	EP3A	190	EG3A	175	EG1B	75

(d) *C. heterophyllum*

	Axis1		Axis 2		Axis 3	
	Sample	Score	Sample	Score	Sample	Score
Highest scoring samples	HN3B	392	HC2A	184	HM4A	201
	HN3A	383	HM4B	174	HS1A	190
	HM1B	377	HC2B	167	HN1A	187
	HM3B	372	HC3B	159	HS2A	186
	HM2A	367	HC4A	140	HP1B	184
	HP2B	363	HN2A	139	HN1B	182
Lowest scoring samples	HN1A	244	HM2A	0	HP2B	69
	HS3B	251	HM2B	32	HC3A	81
	HP3A	252	HS2B	33	HN3B	93
	HN1B	257	HM3B	35	HP3B	95
	HC4B	257	HC1A	42	HC2A	95
	HS2A	259	HM3A	51	HM3B	97

3.4.1 Discussion

Neither the community classifications (Fig 3.1.1) nor the position of samples within the sample ordination (Table 3.2.1) suggest that peripheral populations of *C. acaule*, *C. eriophorum* or *C. heterophyllum* occur in atypical communities.

Both the divisive (TWINSPAN) and the agglomerative (ClustanGraphics) classification methods produce similar clusters of community types. The sample clusters largely reflect the *Cirsium* species for which the site was surveyed, confirming that the species tend to occur in different community types. The exception to this is in the communities of *C. eriophorum* and *C. arvense*, these show greater similarity than the other *Cirsium* communities. The more diffuse grouping of *C. arvense* and *C. eriophorum* sites in the DCA ordination is reinforced by the greater range of community affinities identified by TABLEFIT for the sites surveyed for these species. There is some overlap in the communities occupied by the *Cirsium* species investigated as is evidenced for example by the inclusion of some *C. eriophorum* communities in the clusters of *C. acaule* sites and the communities suggested by TABLEFIT.

Although informative, it is not the main groupings of samples that are of the greatest interest here, rather the outliers from the main groups. In the TWINSPAN classification these are the samples first to split from the clusters and in the ClustanGraphics classification the last to join. *Cirsium acaule*, *C. eriophorum* and *C. heterophyllum* reach distributional limits within the UK whereas *C. arvense* does not. Neither in the classification dendrograms (Fig. 3.1.1) nor in the outlier and intermediate analyses (Table 3.1.1, 3.1.2) are the most remote samples those surveyed within populations found in peripheral areas of the species distribution. The sample ordination confirms this (Table 3.2.1). In all of the data analyses presented here the most remote samples of each species include sites surveyed from across the species range – rather than those surveyed mainly at the species periphery.

In Chapter 2, evidence is presented that supports Brown's (1984) prediction that geographically peripheral populations will be more isolated from one another than those populations occurring in the core areas of a species range. This is explained by a

decline in favourable habitat approaching the species periphery. A further prediction that there will be a decline in the favourability of occupied habitat from core to peripheral areas is not supported, there was little evidence for a decline in the abundance of individuals within peripheral populations.

Lesica and Allendorf (1995) suggest that peripheral populations will often occur in ecologically marginal conditions. They base this on the expected decline in environmental favourability from core to peripheral areas of a species range. They predict that conditions at the periphery are likely to be different even if they are not less favourable and as a consequence many species will occur in novel or atypical habitat at the periphery. Lesica and Allendorf cite Fernald (1925) and Gankin and Major (1964) as supporting this prediction.

Fernald (1925) discusses numerous species that show a major discontinuity in their distribution across the North American continent. Investigating the flora of montane communities in eastern Canada and the north eastern states of the USA, Fernald highlights the presence of species of both arctic and sub arctic distribution and species that are typical of mountain regions of western Canada and the north western United States. The possibility of the recent dispersal of such species from the west is dismissed due to their absence from apparently suitable areas in between. Fernald concludes that these are examples of a relict flora that survived in unglaciated areas during the most recent glaciations. The failure of such species to spread into neighbouring areas following glacial retreat is cited as evidence for their antiquity and poor competitive ability.

Gankin and Major (1964) present an investigation into the California endemic *Arctostaphylos myrtifolia.* This species is limited to extremely acidic and nutrient poor soils that have formed on isolated outcrops of a rare rock type in the Sierra Nevada. Gankin and Major review a large number of examples of unusual plant communities, many of which contain species considered disjunct from their main geographic or edaphic range. In all of the cases they discuss, they relate the presence of such communities to 'peculiar soil parent material'. The communities persist due to the exclusion of the common local vegetation types by the rare edaphic conditions.

The majority of cases described by Fernald (1925) are species that show a major disjunction in their distribution across the USA and Canada, having populations in both eastern and western mountain regions separated by many hundreds of miles. Although such cases are remarkable and the pattern of distribution worthy of investigation, it cannot be said that the eastern localities of these species are geographically peripheral or outlying populations. Fernald describes the regions he later details as being subject to new botanical exploration at the time of writing. Their populations cannot be classed as outliers by virtue of the fact that the eastern localities were the first to be described.

Lesica and Allendorf (1995) draw attention to the important distinction between ecologically marginal and geographically marginal (peripheral) populations. They then predict that peripheral populations will occur in atypical habitats, but base this prediction on evidence from ecologically marginal populations throughout the species range. In most of the cases described by Gankin and Major (1964), the communities occur on a substrate that is considered extremely unusual in its local area. The constituent species in these communities are often formed from species normally occurring on a range of other soil types. The species in these communities are edaphic disjuncts that survive through absence of competition from the typical flora of the area (Billings 1950) – not geographical disjuncts at the edge of the species range.

In apparent support of Lesica and Allendorf's (1995) prediction, Gankin and Major (1964) state that some of these novel communities contain plants that are growing at the edge of their latitudinal or altitudinal range. Whilst this observation cannot be questioned, the converse statement is flawed. It may be the case that the novel communities sometimes contain plants occurring at the edge of their range but it is not accurate to state that 'plants occurring at the edge of their range occur in novel communities' based solely on this evidence. This is a circular argument since it is only these novel communities that have been surveyed.

Despite these criticisms there is some evidence of peripheral populations occurring in novel communities, though it is not possible to say how common this pattern is. Fernald (1921, in Fernald 1925) describes a region of Nova Scotia where typically northern species such as *Carex goodenowii* (Greenland and arctic America, south to Nova Scotia and eastern Massachusetts) occur in the same community as some species

typical of the southern United States such as *Lycopodium inundatum* (Louisiana) and *Utricularia subulata* (Carolina and Louisiana). Similarly, there is mixing of continental and arctic-alpine species in the limestone pavements of North Yorkshire, UK and the 'Burren' of County Clare, Republic of Ireland and in a few other scattered locations in the UK (Pigott and Walters 1954; Pigott 1956). These are rare communities however and frequently composed of species classed as relicts of a pre-glacial flora. It is not possible to comment on potential differences in habitat type without an indication of the communities in which these species occur in other areas of their range.

Perhaps the only convincing piece of evidence comes from Barden (2000), who describes the southern peripheral populations of the common shrub *Quercus ilicifolia.* At its southern limit this species occurs in an uncommon community type known as 'low elevation rocky summit'. This community type is relatively rare and not the typical community type occupied by this species in more central areas of its geographic range.

In attempting to draw general conclusions about differences in the habitat occupied by core and peripheral populations of species it is necessary to use data drawn from a wide variety of species surveyed across their geographic range. It is not sufficient to look only at novel communities when investigating the prediction that peripheral populations will be found in novel or atypical communities. There is little published evidence either in support or contradiction of Lesica and Allendorf's (1995) assertion. The community data presented here suggest that peripheral populations of *Cirsium acaule*, *C. eriophorum* and *C. heterophyllum* do not occur in novel or atypical communities and hence the hypothesis that they will occur in novel or atypical habitat is not supported.

Chapter 4: Clinal variation in plant size and reproductive potential across the latitudinal range of *Cirsium* species

4.1.1 Introduction

It is accepted that climate is the major determinant of plant distribution (Woodward and Williams 1987; Woodward 1990; Pigott 1992). Low temperatures may limit the poleward spread of plant species, acting on both the vegetative and reproductive phases of plant growth (Pigott and Huntley, 1981; Houle and Filion 1993; Woodward 1997). Water availability may limit a species distribution in the equatorial direction (Pigott and Pigott 1993; García et al. 2000) though interspecific competition can play a key role (Woodward 1987).

Investigations into variation within species over geographic ranges have focused on two main areas of study. Firstly, what patterns of trait variation are observed along environmental gradients such as latitude and altitude and what causes such variation? Secondly, what differences are observed between central and peripheral areas of a species range and how can these be explained?

The high degree of ecotypic differentiation in life history traits between core and peripheral populations reported in some studies (Reinartz 1984a; Dorken and Eckert 2001) can complicate comparison between core and peripheral populations since both breeding system and longevity (annual, biennial etc.) may differ. Studies that investigate variation over a gradient including the species periphery may be more informative in indicating causes of variation in ecologically important traits (Jonas and Geber 1999).

Many studies have reported clinal variation in plant characters along altitudinal or latitudinal gradients; for example, the classic study by Clausen, Keck and Heisey (1940) on altitudinal variation in *Achillea landulosa*, showed that plants collected from different altitudes in the Sierra Nevada showed marked variation in height when grown in a common garden. (see also, Pigott 1968; Lacey1984,1988; Aizen and Woodcock

1992; Winn and Gross 1993; Jonas and Geber 1999 for further examples). Although investigation into the effects of reaching a species periphery is often inherent in studies conducted over latitudinal gradients, there is a bias toward investigating the poleward periphery of a species distribution. Studies that record trait variation toward the equatorial periphery or across the entire geographic range of a species are relatively rare (but see Reinartz 1984a; Reinartz 1984b; García *et al.* 2000).

Environmental factors often change in a clinal manner, decreasing in favourability from core to peripheral areas of a species range (Endler 1977; Brown 1984). Habitat at the geographic range margins is thus expected to be ecologically marginal for many species (Lawton 1993; Lesica and Allendorf 1995). A reduction in seed production approaching a species periphery is reported in many studies (eg. Pigott 1968; Pigott and Huntley 1981; García *et al.* 2000; Dorken *et al.* 2001), changes in plant size and morphology are less well studied.

Parsons (1991) suggests decreased environmental favourability at a species periphery should reduce the metabolic energy available for plant growth and reproduction. This is a consequence of the increase in energy demand for repair in a more stressful environment. A reduction in plant size toward the species periphery may result. Variation in plant size with latitude has been reported within a species (Marshall 1968; Clevering *et al.* 2001), together with differential allocation to above and below ground organs (Benowicz *et al.* 2000). Large plant size has been linked to greater reproductive output (Reinartz 1984a; Reinartz 1984b; Primack 1987).

Although a great deal of attention has focused on identifying the environmental factors that limit the current range of many species, there is also much interest in what limits the expansion of a species geographic range over a longer time scale (Bradshaw 1991; Parsons 1991; Hoffmann and Blows 1994). Such information can give important insight into the evolutionary process and the potential response of species distributions to future environmental change.

4.1.2 Materials and methods

Surveys were carried out during July and August 2000 within the populations listed in Table 1.3.1. Population area was estimated by pacing the length and width of the area occupied by each population. For *C. acaule*, population limits were marked on 1:50,000 scale maps and approximate area calculated accordingly. The characters measured in each species are listed in Table 4.1.1. Owing to the local (Derbyshire) red data book status of *Cirsium acaule* and access agreements made with the Peak District National Park Authority (Ecology Service) it was not possible to perform any destructive analysis (eg. biomass) on *Cirsium acaule*, *C. eriophorum* or *C. heterophyllum* in the Peak District National Park. Consequently, with the exception of the collection of seed heads, all measures were entirely non-destructive. Collection of seed heads was restricted to less than 30% of those produced by each Peak District population studied.

Table 4.1.1 Characters measured across the latitudinal range of four *Cirsium* species

Species	Characters measured (n = 30)
Cirsium acaule	Maximum leaf length and width Maximum clump diameter Total filled seed mass per capitulum Percentage of population failing to set seed
Cirsium arvense	Maximum leaf length and width Height at flowering Number of flowers per plant Total filled seed mass per capitulum Percentage of population failing to set seed
Cirsium eriophorum	Maximum leaf length and width Height at flowering Number of flowers per plant Total filled seed mass per capitulum Percentage of population failing to set seed
Cirsium heterophyllum	Maximum leaf length and width Height at flowering Number of flowers per plant Total filled seed mass per capitulum Percentage of population failing to set seed

Vegetative characters

Within each population 30 plants were chosen at random. Major and minor leaf index was measured as the longest length (excluding petiole) and widest width of the youngest fully expanded leaf. Height of the flowering stem was recorded in *C. arvense*, *C. eriophorum* and *C. heterophyllum*. *Cirsium acaule* rarely produces a flowering stem, flowering instead within the rosette. Clump diameter was used as an additional measure of plant growth in this species. The maximum clump diameter was recorded for ten randomly chosen clumps within each population.

The ratio of major leaf index:minor leaf index was used as an indication of leaf morphology whilst the length of the major leaf index indicated leaf size (Mooney and Billings 1961, Rochow 1970). Both these measures together with plant height and clump diameter were regressed against latitude using SigmaPlot 2001 for Windows v7.

Reproductive characters

30 flowering individuals from each population were selected at random. The number of flower heads (capitula) per plant was recorded for *C. arvense*, *C. eriophorum* and *C. heterophyllum*. The total number of capitula produced per plant was not recorded for *C. acaule* as the indeterminate flowering of populations of this species within a season would require extended monitoring of each population. To assess seed production, 30 ripe but not dehiscent capitula were collected at random within each population. In *Cirsium arvense* these were chosen only from female flower heads (Moore 1975; Heimann and Cussans 1996), in *C. eriophorum* and *C. heterophyllum* the terminal capitulum was selected from the flowering stem of each plant.

Seeds (achenes) within the capitulum of *Cirsium* species usually fall into one of three groups. Viable seeds when ripe are usually filled by the embryo and are swollen and hard when soaked in water. Sterile seeds fall into two groups: either they are shrivelled and apparently contain no embryo, or the pericarp is fully grown but the embryo fails to develop – such seeds collapse when squeezed after soaking in water (Pigott 1968). Tests showed it was also possible to differentiate between viable and sterile but non-shrivelled seeds on the grounds of appearance. Filled seeds were shiny and dark in

colour whereas those lacking an embryo tended to be dull and much lighter in colour than filled seed from the same capitulum. In uncertain cases squeezing seed with forceps could be used to differentiate between filled and non-filled seeds.

Capitula were air dried in paper envelopes for one month prior to de-seeding. Seeds were extracted from each capitulum by removing the papus material and seeds from each capitulum and rubbing this across a soil sieve (4 mm mesh). The collected seeds were then cleaned of debris and sorted by hand using a hand lens to separate filled from non-filled seeds. It was not possible to separate shrivelled seeds from debris as these frequently disintegrated during the de-seeding procedure.

A number of insect larvae are known to predate the seeds of *Cirsium* species (Pigott 1968; Moore 1975; Klinkhammer and de Jong 1993; Heimann and Cussans 1996; Tofts 1999). Damage is usually identifiable by a small hole in the seed coat or its complete fragmentation. Predation of this sort was low in all species other than *C. eriophorum*. In *C. eriophorum* capitula were given a score of 1 to 4 to indicate estimated levels of predation. 1 = negligible or no observable predation, 2 = less than 33% predation, 3 = 33% - 66% predation and 4 = 66% - 100%. Clearly these are approximate and based on a visual inspection of the quantity of remains of predated seeds compared with the remaining unpredated seeds. Those capitula containing no filled seeds and with no evidence of predation were scored 0.

Total filled seed mass per capitulum was recorded (Mettler Toledo AG245 balance, Mettler Toledo, Beaumont Leys, Leicester, UK). In all species a seed mass of 0 was recorded for those capitula containing no filled seeds and with no evidence of predation. This was assumed to indicate those individuals that failed to set seed.

In *C. eriophorum* the estimate of percentage seed predation per capitulum was used to calculate the estimated seed mass lost due to predation from the measurement of the remaining seed mass. This estimate was then added to the total seed mass per capitulum to estimate seed production. Capitula suffering total seed predation were excluded. Seed mass was adjusted by the mid point of the predation estimate range, eg. capitula suffering 0 – 33% predation were deemed to have lost 16.5% of their seed mass.

For each species, mean total seed mass per population and the percentage of each population failing to set seed (and in *C. eriophorum,* % predation of each population), was regressed against latitude using SigmaPlot 2001 for Windows v7.

4.1.3 Results

Vegetative characters

In *Cirsium acaule* there is a significant linear relationship between clump diameter and latitude ($R^2 = 0.53$, $P < 0.01$). Clump size is smallest (25.5 cm) in the core populations of this species and increases toward the periphery (48.5 cm). A single population mid way across the range (population ABA1) reaches an unusually large clump size of 51 cm, this is slightly larger than the maximum clump size found in peripheral populations in the Peak District (Fig. 4.1.1). There is no relationship between any other vegetative character measured and latitude in any species (for summary data see Appendix C).

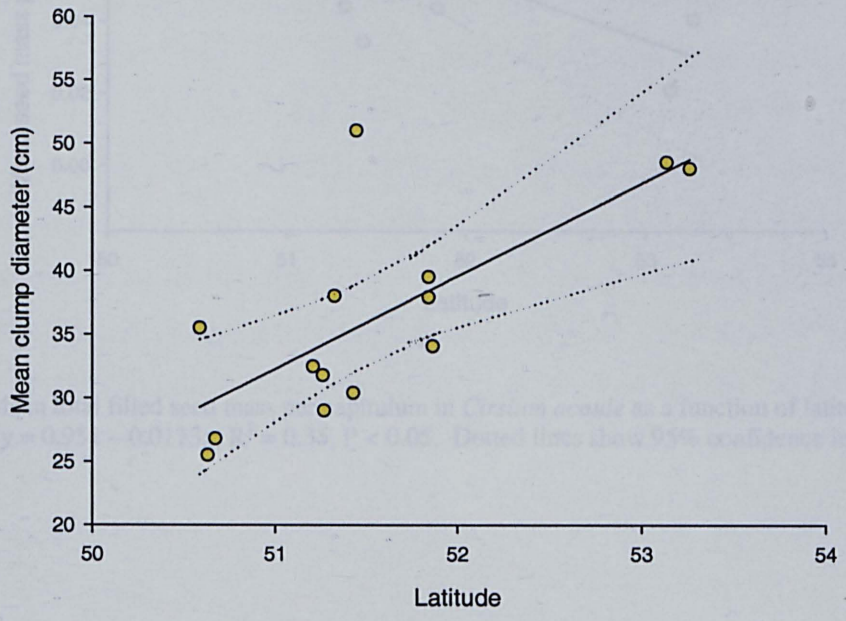

Fig. 4.1.1 Mean clump diameter per population in *Cirsium acaule* as a function of latitude. Regression: $y = -338 + 7.26x$, $R^2 = 0.53$, $P < 0.01$. Dotted lines show 95% confidence interval of regression

Reproductive characters

There is a significant relationship between seed production and latitude in both *C. acaule* ($R^2 = 0.35$, $P < 0.05$) and *C. heterophyllum* ($R^2 = 0.51$, $P < 0.01$). In both species seed mass per capitulum declines approaching the periphery. This decline is most dramatic in *C. heterophyllum*. At its southern periphery this species produces only 1.2% of the maximum seed mass recorded in the core area of its UK distribution (Fig. 4.2.2). Maximum seed production in *C. acaule* at its northern periphery is 37% of the maximum recorded in the core area of its distribution in southern England (Fig. 4.2.1).

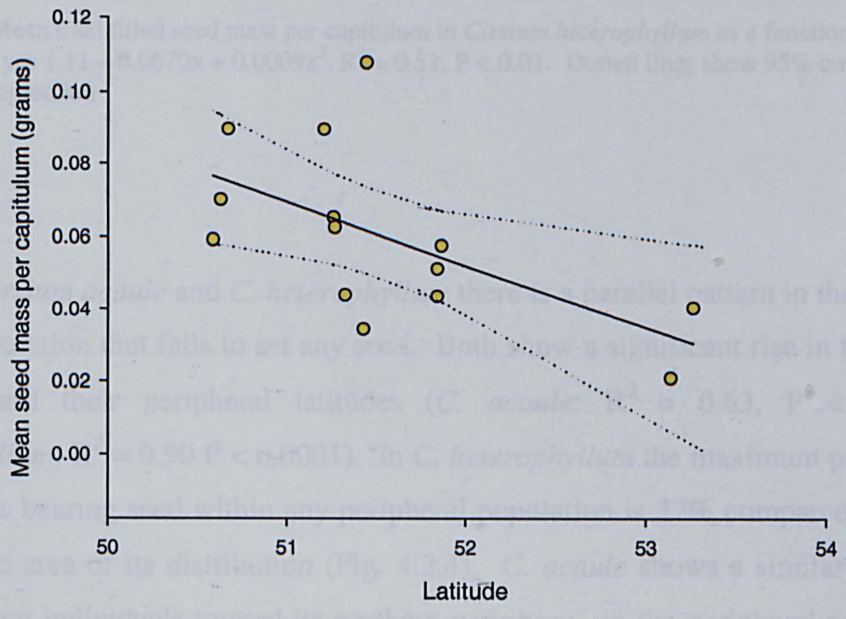

Fig. 4.2.1 Mean total filled seed mass per capitulum in *Cirsium acaule* as a function of latitude. Regression: $y = 0.951 - 0.0173x$, $R^2 = 0.35$, $P < 0.05$. Dotted lines show 95% confidence interval of regression

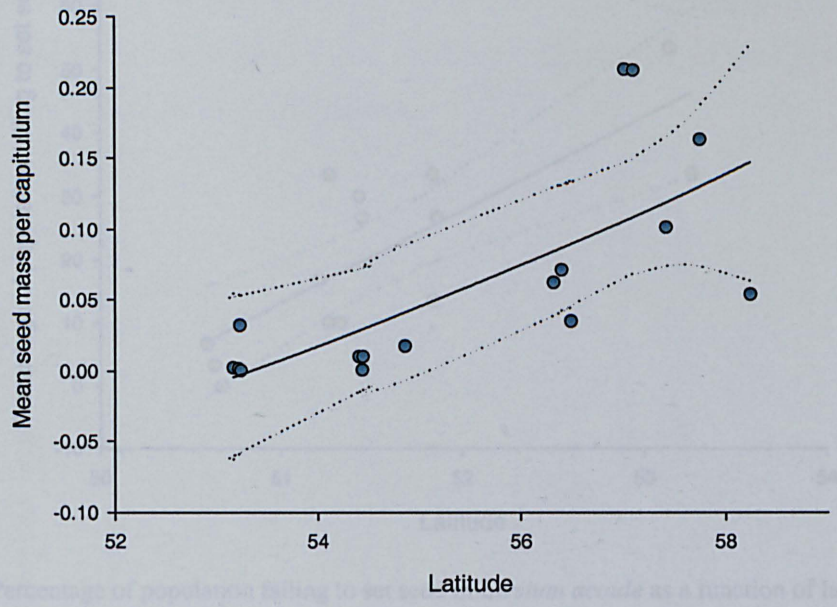

Fig. 4.2.2 Mean total filled seed mass per capitulum in *Cirsium heterophyllum* as a function of latitude. Regression: $y = 1.11 - 0.0670x + 0.0009x^2$, $R^2 = 0.51$, $P < 0.01$. Dotted lines show 95% confidence interval of regression

In both *Cirsium acaule* and *C. heterophyllum* there is a parallel pattern in the percentage of the population that fails to set any seed. Both show a significant rise in failure to set seed toward their peripheral latitudes (*C. acaule*: $R^2 = 0.63$, $P < 0.001$; *C. heterophyllum*: $R^2 = 0.90$ $P < 0.0001$). In *C. heterophyllum* the maximum percentage of individuals bearing seed within any peripheral population is 37% compared with 100% in the core area of its distribution (Fig. 4.2.4). *C. acaule* shows a similar decrease in seed bearing individuals toward its northern periphery, in the peripheral populations a maximum of 47% of individuals were found to set seed as opposed to a maximum of 100% in core populations (Fig. 4.2.3).

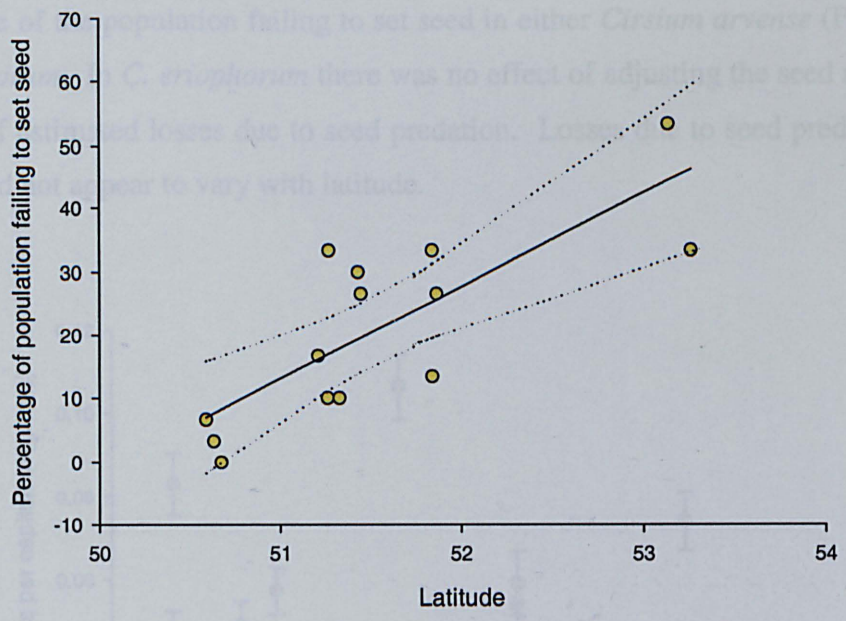

Fig. 4.2.3 Percentage of population failing to set seed in *Cirsium acaule* as a function of latitude. Regression: $y = -733 + 14.6x$, $R^2 = 0.63$, $P < 0.001$. Dotted lines show 95% confidence interval of regression

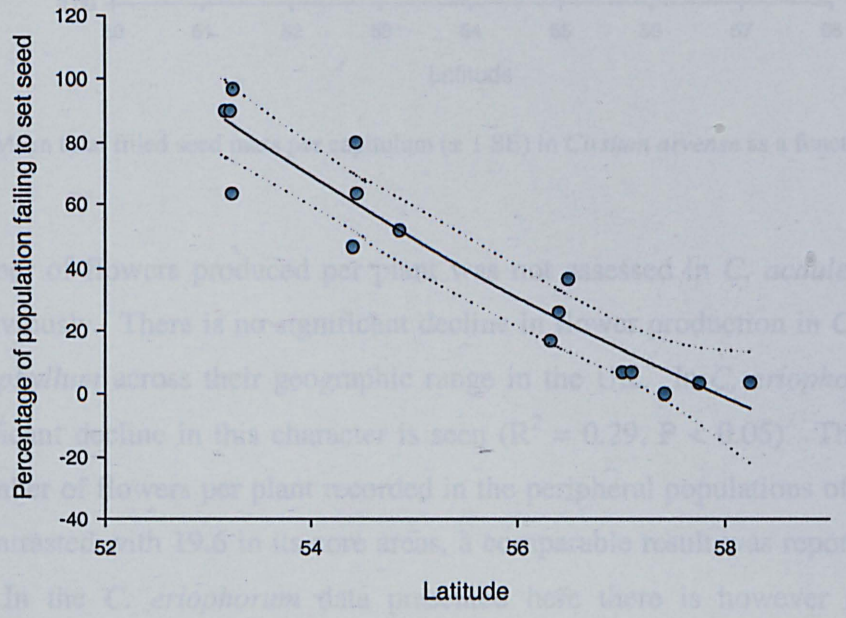

Fig. 4.2.4 Percentage of population failing to set seed in *Cirsium heterophyllum* as a function of latitude. Regression: $y = 3596 - 110x + 0.823x^2$, $R^2 = 0.90$, $P < 0.0001$. Dotted lines show 95% confidence interval of regression

There is no relationship with latitude in either mean seed mass per capitulum or the percentage of the population failing to set seed in either *Cirsium arvense* (Fig. 4.2.5) or *C. eriophorum*. In *C. eriophorum* there was no effect of adjusting the seed mass to take account of estimated losses due to seed predation. Losses due to seed predation in this species did not appear to vary with latitude.

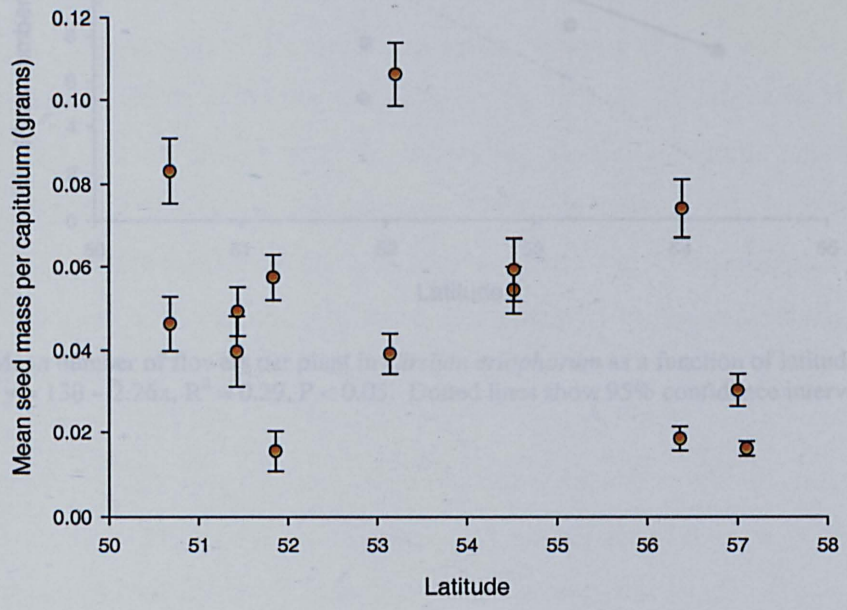

Fig. 4.2.5 Mean total filled seed mass per capitulum (± 1 SE) in *Cirsium arvense* as a function of latitude

The number of flowers produced per plant was not assessed in *C. acaule* for reasons stated previously. There is no significant decline in flower production in *C. arvense or C. heterophyllum* across their geographic range in the UK. In *C. eriophorum* a weak but significant decline in this character is seen ($R^2 = 0.29$, $P < 0.05$). The maximum mean number of flowers per plant recorded in the peripheral populations of this species is 7.3, contrasted with 19.6 in its core areas, a comparable result was reported by Tofts (1999). In the *C. eriophorum* data presented here there is however considerable dispersal of the values for mean number of flowers per plant around the regression line; the lowest value of 5.2 and was recorded toward the core area of its UK distribution (Fig. 4.2.6).

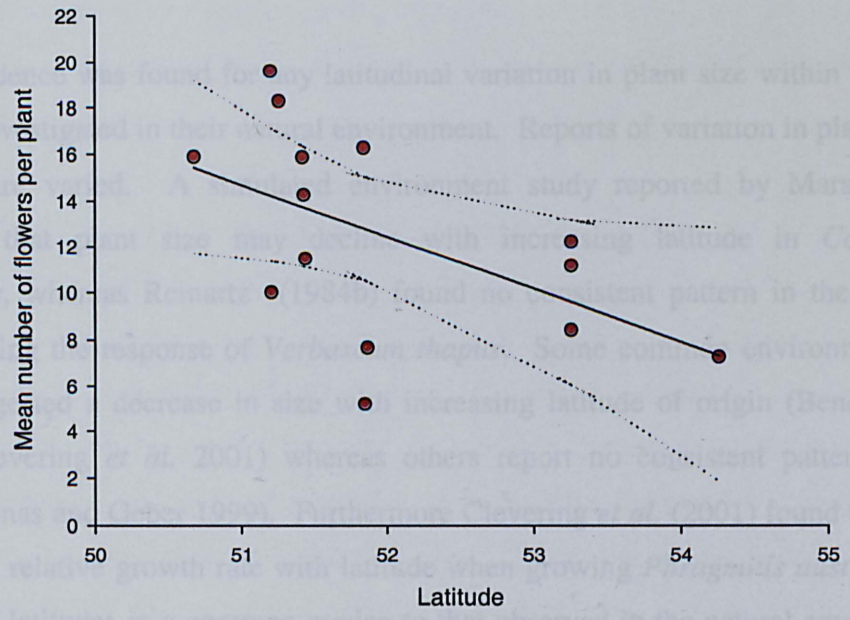

Fig. 4.2.6 Mean number of flowers per plant in *Cirsium eriophorum* as a function of latitude. Regression: $y = 130 - 2.26x$, $R^2 = 0.29$, $P < 0.05$. Dotted lines show 95% confidence interval of regression

4.1.4 Discussion

Little evidence was found for any latitudinal variation in plant size within the *Cirsium* species investigated in their natural environment. Reports of variation in plant size with latitude are varied. A simulated environment study reported by Marshall (1968) suggests that plant size may decline with increasing latitude in *Corynephorus canescens*, whereas Reinartz (1984b) found no consistent pattern in the field when investigating the response of *Verbascum thapus*. Some common environment studies have suggested a decrease in size with increasing latitude of origin (Benowicz *et al.* 2000; Clevering *et al.* 2001) whereas others report no consistent pattern (Reinartz 1984b; Jonas and Geber 1999). Furthermore Clevering *et al.* (2001) found the opposite pattern in relative growth rate with latitude when growing *Phragmitis australis* from a variety of latitudes in a common garden to that observed in the natural environment by Čížková (1999, in Clevering *et al.* 2001).

In the *Cirsium* species investigated, there is little evidence of clinal variation in plant size with latitude in the natural environment. The diversity of responses reported in the literature suggests that although genetic differences may exist both in potential growth rate and plant size, these may not necessarily be realised under the conditions occurring in populations at the plant's latitude of origin.

Parsons (1991) proposed that environmental stress levels will rise toward a species periphery and that a consequence of this will be a decrease in energy available for growth and reproduction. The geographical limit at which a species may grow and that at which it may produce viable seed may be determined by different environmental conditions (Woodward 1997) and sometimes widely separated in space (Woodward 1990). At geographic limits where species can grow and complete a normal life cycle successful regeneration is not guaranteed even if viable seed is produced; thus the realised range of a species may differ from that which it might theoretically occupy based on individual survival (Pigott 1992). The proposed effect of stress might not be seen either on plant growth or reproduction if a species distribution is limited by failure to set seed. If the stress effect occurs as a result of a reduction in metabolic energy availability as Parsons suggests, the decline in reproduction would be mediated by the

whole plant response to stress. Since due to reproductive failure, the plant would be absent from the region Parsons expects to be highest in stress, the proposed effects of stress may not be detectable as a reduction in growth or reproduction in the natural environment.

The significant increase in clump size approaching the periphery of C *acaule* is the only vegetative character that showed a relationship with latitude (Fig. 4.1.1). It is doubtful however that clump size can be compared with plant height in the other *Cirsium* species investigated as a measure of plant size. Clump size increases annually through extension and branching of the rhizome of *C. acaule*, thus although there is an apparent increase in genet size with latitude, the ramet size (as indicated by the leaf size and morphology measurements) remains the same. Clump size (presumed clone size) may therefore indicate the extent of vegetative reproduction (Pigott 1968) and is not comparable with investigations of plant size studies reported above, all of which discuss ramet size. This pattern and the implications of increased vegetative reproduction approaching the species geographical limit will be discussed in Chapter 6.

Evidence was found for declining reproductive potential approaching the species periphery in each of the species reaching a geographical limit in the UK. Both *Cirsium acaule* and *C. heterophyllum* show a significant decline in seed production toward the periphery of their distribution (Fig. 4.2.1, 4.2.2). This pattern is absent in *C. eriophorum*, which instead shows a decline in the number of flowers produced toward its northern limit (Fig. 4.2.6). Both *C. acaule* and *C. heterophyllum* show a major increase in the percentage of the population failing to set seed as the periphery is approached (Fig. 4.2.4, 4.2.5), this pattern was not detected in *C. eriophorum*. Repetition of this survey in successive years would be necessary to determine the effect that inter-annual variation in climate may have on these patterns.

Declining seed production approaching the poleward periphery of a species distribution has been reported in numerous studies (Pigott 1968; Pigott and Huntley 1981; Eckert and Barrett 1993; García *et al.* 2000; Dorken and Eckert 2001). Reinartz (1984b) and García *et al.* (2000) describe lower seed production at the equatorial periphery but only García *et al.* (2000) present evidence for a clinal decrease. Although fertilisation failure has been reported (Pigott and Huntley 1981), seed set may be poor even when

successful fertilisation occurs (Pigott 1968; Stephenson 1981). Failure of the *Cirsium* species investigated here to set viable seed is unlikely to be due to a failure of successful fertilisation since all share common pollinators and floral characteristics with widespread species that set viable seed throughout their UK latitudinal range (Pigott 1968; Klinkhammer and de Jong 1993; Heimann and Cussans 1996; Tofts 1999). In both *Cirsium acaule* and *C. heterophyllum*, the decline in seed production per population (as measured by total seed mass per capitulum) is paralleled by a rise in the percentage of the population failing to set seed. The reproductive potential of these species via seed in their peripheral areas is therefore markedly reduced.

Although there is no simple relationship between the amount of seed produced and the success of regeneration (Pigott 1992), the extremely low seed production of *C. heterophyllum* at its southern periphery suggests regeneration by seed in these populations is unlikely. The probability of regeneration by seed of *C. acaule* at its northern periphery is likewise much reduced. *Cirsium acaule*, *C. arvense* and *C. heterophyllum* may reproduce vegetatively, producing new shoots from the rhizome; this method of reproduction is absent in *C. eriophorum*. Populations of long-lived polycarpic perennial species such as *C. acaule* and *C. heterophyllum* may persist even in the absence of seed set (Pigott 1992). The monocarpic *C. eriophorum* however must produce sufficient quantity of viable seed for a population to persist; the less pronounced decline in the reproductive potential of this species approaching its northern periphery is therefore not surprising.

The upper limit on seed production during a reproductive period is a function of the number of flowers produced and the number of ovules within each flower. The fraction of this maximum that is realised will depend on the number of pollinated flowers, the number of fertilised ovules, fruit and seed predation, weather conditions and resource provision by the maternal plant (Stephenson 1981). Pollen limitation is unusual and many species regularly abort a large proportion of immature seeds (Stephenson 1981; Charlesworth 1989; Kärkkäinen *et al.* 1999).

Explanations for seed abortion have been proposed based on the genetic characteristics of both seed and the maternal plant. These include early selective abortion of offspring carrying high genetic load (the reduction in fitness due to the accumulation of

deleterious mutations) – so called 'bad offspring' (Wiens *et al.* 1987). An alternative though not exclusive explanation is abortion due to high genetic load carried by the maternal plant – the 'bad maternal genotype' (Kärkkäinen *et al.* 1999). Wiens *et al.* (1987) suggest that 'developmental selection' may also contribute to seed abortion. They use this term to describe hypothesised competition amongst developing ovules, with the least vigorous ovules being aborted. Stephenson (1981) and Charlesworth (1989) suggest seed abortion levels probably indicate the availability of resources for seed maturation, this they refer to as 'resource limitation'.

Resource limitation, developmental selection and poor maternal or offspring genotype are not mutually exclusive theories. Wiens *et al.* (1987) state that genetic load and developmental selection provide the best explanation for seed abortion and give lesser importance to resource limitation. However, Stephenson (1981) argues that resource limitation should reduce fruit production in most cases.

Under resource limitation, pollinated flowers and juvenile fruits abscise until seed number matches the available resources (Stephenson 1981). Resource limitation allows for both poor seed set in unfavourable years and enhanced seed set in those better than average – a pattern that is frequently correlated with variation in climate (Pigott, 1968, Pigott and Huntley 1981, Houle and Filion 1993; Despland and Houle 1997; Woodward 1997). Wiens *et al.* (1987) state that such fluctuations in reproductive output are likely to be minor variations within the pattern dictated by genetic load or developmental selection, yet it is difficult to interpret the studies cited above in this way. In long-lived perennial species (including *C. acaule*, *C. arvense* and *C. heterophyllum*) the array of maternal genotypes within a population is not likely to change dramatically between years. Likewise since most pollination will result from within population rather than between population transfer it is unlikely that maternal or offspring genotypes are likely to become 'bad' suddenly, thereby prompting mass abortion.

Resource limitation might better explain variation in seed production within populations that is associated with yearly fluctuations in climate. Whilst it seems unlikely that there will be a clinal increase in bad genotypes (whether maternal or offspring) approaching the periphery of both *C. acaule* and *C. heterophyllum* this cannot be discounted by these

data. (Genetic characteristics of the *Cirsium* populations studied are presented in Chapter 5). A number of studies report an increase in sexual reproduction in northern peripheral populations that coincides with unusually warm years (Pigott 1968; Pigott and Huntley 1981; Pigott 1992; Houle and Filion 1993). Variation in reproductive output with climate suggests bad genotype effects are less likely to explain the latitudinal pattern in seed production than resource limitation for the reasons outlined above.

If resource availability is the primary determinant of seed production in many species as Stephenson (1981) suggests, it is likely that genetic load and developmental selection may well determine which embryos are aborted. This might be particularly important considering the rise in the percentage of the population failing to set seed approaching the periphery of *Cirsium acaule* and *C. heterophyllum* which suggests a major difference in the ability of individuals to produce seed in their given environment. García *et al.* (2000) suggest genetic load may be particularly important in elevating abortion rates in isolated populations of *Juniperus communis*. Since populations of the *Cirsium* species investigated become more isolated as the species periphery is approached (Chapter 2), increased genetic load could play an important role in further reducing seed production in peripheral populations of these species.

Numerous studies suggest that the reproductive phase of many plants is particularly sensitive to climate (Marshall 1968; Pigott 1968; Pigott and Huntley 1981, Houle and Filion 1993; Despland and Houle 1997; Woodward 1997; García *et al.* 2000). If reproduction rather than vegetative survival limits the distribution of many plant species, the hypothesised reduction in plant growth at the periphery of a species distribution may not be detected in the natural environment. The three *Cirsium* species investigated here that reach a geographical limit in the UK show a decline in reproductive potential by seed toward their periphery. This is most likely to occur through resource limitation as suggested by Stephenson (1981) and may be explained in part by the decline in environmental favourability predicted by Brown (1984). Although the increase in stress predicted by Parsons (1991) might enhance seed abortion levels, this would be difficult to detect above direct climatic limitations reported by some authors (for example: Pigott 1968; Pigott and Huntley 1981). The failure of an increasing proportion of the population of *C. acaule* and *C. heterophyllum*

to set seed approaching the range edge presents an interesting prospect for further investigation given the effects of genetic load proposed by Weins *et al.* (1987) and Kärkkäinen *et al.* (1999).

Chapter 5: Geographic patterns in genetic variation within *Cirsium* species

5.1.1 Introduction

Low temperatures during Quaternary glaciations are thought to have eliminated many of the species present in northern Europe. Species expanded from glacial refugia as the climate warmed and contracted into refugia or became extinct as the climate cooled (Pigott and Walters 1954; Godwin 1975). Migration following the most recent glaciation is thought to have heavily influenced the current day distribution of genetic variation within species (Taberlet *et al.* 1998; Hewitt 2000). Continuing processes such as gene flow and genetic drift will modify this pattern dependent on the size and isolation of populations (Lesica and Allendorf 1992, 1995) and a species' ecological characteristics (Loveless and Hamrick 1984; Hamrick and Godt 1996). In the absence of major changes to distribution brought about by man, the poleward and equatorial periphery of a species' current geographic range will represent the leading and retreating edge of many species' post-glacial migration (Hewitt 2000). Consideration of both the current distribution of populations and the likely post glacial history of a species is necessary when attempting to understand the present distribution of genetic variation within a species.

Geographically peripheral populations are often expected to be smaller and more isolated than those in more central areas of a species' range (Brown 1984; Lesica and Allendorf 1995). As geographic isolation increases, a reduction in both seed dispersal and pollen flow will result in decreased gene flow between populations (Ellstrand and Hoffman, 1990; van Dorp *et al.*, 1996). The resulting genetic isolation of populations may lead to pronounced geographic structuring in genetic variation within a species (Lesica and Allendorf 1995) as peripheral populations are likely to be isolated both from the core of the species' range and from each other. Reduced dispersal (seed flow) from neighbouring populations may also result in demographic instability in isolated populations (Schaal and Leverich 1996) with the potential to induce genetic bottlenecks at the periphery (Lesica and Allendorf 1995).

Both population size and isolation will affect levels of genetic variation within populations and geographical structure of genetic variation within a species. Reduced size and increased genetic isolation may result in lower levels of genetic variation within a population (Raijmann *et al.* 1994; Schaal and Leverich 1996; Lammi *et al.* 1999). However, theoretical predictions regarding the effects of reduced population size are not always supported by the data (Ellstrand and Elam 1993), and there is some evidence that selection favouring heterozygotes may increase diversity in peripheral populations (Lesica and Allendorf 1992, 1995). The effects of variation in population size and isolation will vary with characteristics such as plant longevity, mating system and population history (Barrett and Kohn 1991; Ellstrand and Elam 1993; Young *et al.* 1996; Newman and Pilson 1997).

In small populations, allele frequencies may undergo large and unpredictable changes due to genetic drift - the random change in allele frequency as a consequence of gametes from one generation only transmitting a proportion of the alleles present within the population to the next generation (Ellstrand and Elam 1993). Genetic drift will have a dominant influence in small and isolated populations, resulting in decreased genetic variation and increased population differentiation. Variation in such populations is lost more readily than in populations where drift is not a major factor (Barrett and Kohn 1991; Ellstrand and Elam 1993).

Levels of inbreeding (the mating of related individuals) are also likely to rise as population size decreases and isolation increases (Schaal and Leverich 1996). Although mating systems are the prime determinants of the levels of inbreeding in plants, populations become inbred more rapidly when they are of small size and do not receive new alleles through gene flow (Barrett and Kohn 1991; Schaal and Leverich 1996). Small population size may overwhelm the effect of plant mating system in its contribution to inbreeding in plants, although inbreeding is likely to be higher if a species has high levels of self-fertilisation (Barrett and Kohn 1991).

Both genetic drift and inbreeding can affect fitness through inbreeding depression – the relative reduction in fitness of inbred offspring compared with outcrossed offspring (Barrett and Kohn 1991). Inbreeding depression may result from increased

homozygosity within populations and the expression of deleterious recessive alleles (Hartl 1988). Inbreeding depression has been linked to reduction in many components of plant fitness (Karron 1989; Menges 1991; Ouborg *et al.* 1999; Oostermeijer *et al.* 1995) and ultimately population persistence (Newman and Pilson 1997). Inbreeding effects are often most pronounced late in a plant's life cycle, with flower and fruit production being particularly sensitive (Barrett and Kohn 1991).

Genetic diversity may be reduced as a result of founder effects (sequential genetic subsampling) during colonisation of an area (Stone and Sunnucks 1993; Durka 1999; Lammi 1999). Founder effects are likely to result in declining diversity with distance from core or refugial areas during species' migration (Stone and Sunnucks 1993; Dumolin-Lapègue *et al.* 1997; Taberlet *et al.* 1998); thus diversity may decline approaching a species' range limits irrespective of the effects of population size and isolation.

Genetic isolation of geographically isolated populations may be important in permitting adaptation to environmental conditions at the range boundary (Safriel *et al.* 1994), although isolation from core populations has the potential to disrupt long-term adaptation due to the paucity of supply of new genetic variation (Schaal and Leverich 1996). Divergent natural selection at the species periphery may promote differentiation of populations (Safriel *et al.* 1994) in addition to the effects of genetic stochasticity (i.e., inbreeding and genetic drift) outlined previously. Geographically peripheral populations are considered to be important for the evolutionary potential of the species and the site of future speciation events (Lesica and Allendorf 1995 and references therein); they may be of key importance in a species' response to environmental change (Safriel *et al.* 1994).

Much effort is devoted to conserving peripheral populations on the grounds that they are presumed to be genetically distinct (Lesica and Allendorf 1995). However, the genetic structure of peripheral populations results from the complex interaction between a species' history and genetic and demographic stochasticity. This chapter aims to discover whether present day patterns in the geographic distribution of populations are reflected in predicted parallel patterns of genetic variation. Specifically, it will test the assumptions that genetic variation will decline approaching a species' range boundary

and that peripheral populations will be genetically divergent both from each other and populations in more central areas of the species' range.

5.2.1 Materials and methods

Microsatellites consist of tandemly repeated units of short nucleotide repeats that are 1-6 base pairs (bp) long (e.g., $(CA)_n$, $(AAT)_n$, $(GCTC)_n$). There is great variability in the number of repeat units between individuals and hence the length of the microsatellite (Peakall *et al.* 1998; Hancock 1999). The extreme polymorphism of microsatellite markers makes them ideal markers for use in conservation biology and population genetics (Peakall *et al.* 1998 and references therein; Beaumont and Bruford 1999).

DNA sequence knowledge is necessary to allow the development of appropriate primers for polymerase chain reaction (PCR) assay of microsatellites, hence their development is expensive and time consuming. Cross-species amplification of loci amongst closely related species is usually possible (see for example, Peakall *et al.* 1998; Cipriani *et al.* 1999; Di Gaspero *et al.* 2000; Roa *et al.* 2000). Of the *Cirsium* species investigated here, *Cirsium acaule* yielded the highest quality DNA. Consequently, microsatellite loci were developed in this species. Loci used in the following analyses were selected following the screening of the *Cirsium acaule* primers to identify those that would also amplify polymorphic loci in other *Cirsium* species.

5.2.2 Extraction and purification of DNA

Total genomic DNA was extracted from fresh *Cirsium acaule* leaves following Doyle and Doyle's (1987) protocol. 1g of fresh tissue was ground in liquid nitrogen and added to 10 ml CTAB extraction buffer (2%CTAB (hexadecyltrimethylammonium bromide), 1.4 M NaCl, 20 mM EDTA (pH8), 100 mM Tris (pH8), 2% PVP-40 (polyvinyl pyrrolidone)) at 65°C. 100 μl of β mercaptoethanol was added and the mixture shaken briefly and incubated at 65°C for 20 min. An equal volume of 25:24:1 phenol:chloroform:isoamyl alcohol was added, the mixture was mixed by inversion for 5 minutes and then centrifuged at 14000 rpm for 10 min. The supernatant was removed

to a new sterile tube and the phenol:chloroform:isoamyl alcohol extraction repeated until the interface between the two phases was clean. The supernatant was removed to a new sterile tube and an equal volume of isopropanol (-20°C) was added. The mixture was inverted and then centrifuged at 14000 rpm for 20 minutes. The liquid was drained from the pellet and the pellet washed in 300 μl 70% ethanol (-20°C). The drained pellet was air dried overnight.

The pellet was resuspended in 300 μl sterile distilled water containing 10 ng/ul RNAse (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK) and incubated at 37°C for 1 hour. The phenol:chloroform:isoamyl alcohol extraction was repeated until the interface was clean. The supernatant was removed to a new sterile tube, 10% volume of 3 M sodium acetate was added and 100% volume isopropanol (-20°C). The mixture was then centrifuged at 14000 rpm for 20 minutes and the pellet drained, washed and dried as before.

The cleaned, RNA free pellet was resuspended in 300 μl sterile distilled water and quantified by fluorometer following the manufacturers instructions (Hoeffer DyNA Quant 200, Amersham Pharmacia Biotech).

5.2.3 Microsatellite library development and primer design

Summary of molecular methods:

DNA extracted and purified from fresh leaves taken from two individuals of *Cirsium acaule* was used to develop the microsatellite library. Library development and successive stages leading to the design of primers are outlined below and detailed in the following protocols.

Section 1) Development of the microsatellite library

Digestion of genomic DNA – to produce fragments of a size suitable for cloning.
Ligation of adapters to the ends of the DNA fragments to provide primer binding sites for PCR and restriction sites for cloning.

Pre-enrichment amplification of the fragments by polymerase chain reaction (PCR) to increase the number of fragments and select out the fragments without adapters.
Enrichment – selection of fragments by hybridisation to a membrane bound with microsatellite oligonucleotides of interest.
Denaturing and elution of the selected fragments from the membrane.
Post-enrichment amplification of the fragments to make them double stranded and their subsequent digestion to remove adapters.
Ligation into a plasmid vector.

Section 2) Pre-sequencing duplication of microsatellites

Transformation into *Escherichia coli* cells.

Section 3) Detection and characterisation of microsatellites

Screening of transformed bacteria using ^{32}P radiolabled probes to identify those containing microsatellites.
Sequencing of transformants to determine microsatellite sequence and allow primer design.

Section 4) sequence comparison and primer design

Sequence comparison to ensure sequences are unique.
Primer design to enable PCR amplification of microsatellites from genomic DNA .

Protocols in section 1 describe methods used routinely at Horticultural Research International East Malling (HRI), West Malling, Kent, UK, originally derived from the method of Edwards *et al.* (1996) with subsequent modifications by C.M. James, HRI. Sections 2 and 3 describe methods used routinely at the Natural Environment Research Council Sheffield Molecular Genetics Facility (SMGF) at the University of Sheffield, UK. These methods are based on those of Buluwela *et al* (1989) and Feinberg and Vogelstein (1983) with modifications made by D.A. Dawson, SMGF.

Protocols:

Section 1) Developing the microsatellite library

The following protocol describes the development of an enriched microsatellite library from total genomic DNA.

Preparation of enrichment filters. The following oligonucleotides were used to enrich for DNA fragments containing microsatellites:
$[GC]_{20}$, $[AC]_{20}$, $[GA]_{20}$, $[GT]_{20}$, $[CAA]_{14}$, $[GCC]_{14}$, $[CTG]_{10}$, $[CAG]_{10}$.
50 ng of each oligonucleotide in 3× standard saline citrate buffer (SSC) (45 mM sodium citrate, pH 7.0 and 450 mM NaCl) were pooled in a total volume of 80 μl. The pooled oligonucleotides were denatured by boiling for five minutes and immediately placed on ice before being spotted onto a 1 cm^2 piece of Hybond N+ (Amersham, Arlington Heights. IL. USA) and air-dried for 1 hour. The dry membrane was foil wrapped, baked for 2 hours at 80°C, then UV-treated for 60 s using a 260-nm transilluminator. Weakly bound oligonucleotides were washed off the membrane by washing twice in 10 ml of hybridisation buffer (50% formamide, 3× SSC, 25 mM $NaHPO_4$ (pH 7.0) and 0.5% sodium dodecyl sulfate (SDS)) at 45°C for two days. The membranes were stored at -20°C until required.

Digestion of genomic DNA and ligation of adapters. Approximately 200 ng DNA was digested with 5 units of *Rsa*1 (Amersham Pharmacia Biotech) and 1× reaction buffer in a volume of 50 μl for 3 h at 37°C. 50 ng of an *Mlu*1 adaptor (consisting of a 21-mer: 5'CTCTTGCTTACGCGTGGACTA3' and a phosphorylated 25-mer: 5'pTAGTCCACGCGCTAAGCAAGAGCACA3'; Edwards *et al.* 1996) together with 0.5 μl 100 mM ATP was added to the digestion mixture with 1 unit of T4 DNA ligase (Amersham Pharmacia Biotech). Ligation was then allowed to proceed for 2 h at 37°C.

Pre-enrichment amplification. Undiluted ligation mix was used as a template for pre-amplification, three replicates were carried out and combined. 2 μl DNA template was amplified by PCR using 400 ng of the 21-mer adapter primer (5'CTCTTGCTTACGCGTGGACTA3'; Edwards *et al.* 1996) in 100 μl. Reaction

conditions were 1.5 mM $MgCl_2$, 200 μM each dATP, dCTP, dGTP,and dTTP, 1× reaction buffer and 2.5 units Taq DNA Polymerase (Amersham Pharmacia Biotech). Amplification was allowed to proceed for 20 cycles (95°C for 40 seconds, 60°C for 60 seconds and 72°C for 180 seconds; PTC-100, MJ Research inc., Waltham, MA, USA).

Phenol cleanup and ethanol precipitation. PCR products were pooled and extracted with 300 μl of phenol. The mixture was centrifuged at 16000 rpm for 10 min. The supernatant was extracted with 300 μl chloroform and centrifuged at 16000 rpm for 10 min. The supernatant was made up to 100 mM NaCl and -20°C ethanol was added equal to the total volume. The mixture was then incubated on ice for 1 hour before being centrifuged at 16000 rpm for 20 min. The supernatant was discarded and the DNA pellet air dried then resuspended in 300 μl sterile distilled water.

Enrichment. The cleaned amplified DNA was then enriched for microsatellites.
50 μl of DNA was denatured by boiling for 5 min and added to 1 ml hybridisation buffer containing 2 μg of the 21mer oligonucleotide and one Hybond N^+ filter with bound oligonucleotides. Hybridisation proceeded for 24 h at 50°C with gentle shaking.

Elution. Following hybridisation, the filter was washed three times for 5 min in 2× SSC, 0.01% SDS at 60°C, followed by three times for 5 min in 0.5× SSC, 0.01% SDS at 60°C. Bound DNA was then denatured and recovered from the filter by boiling for 5 min in 500 μl sterile distilled water. The elutate was brought to 100 mM NaCl and ethanol precipitated as before. Following drying, the pellet was re-suspended in 25 μl sterile distilled water.

Post-enrichment amplification. The enriched fraction was then amplified as before, using 4 μl of the eluted, microsatellite enriched DNA as a template. The PCR programme consisted of 25 cycles of (95°C for 40 seconds, 60°C for 60 seconds and 72°C for 180 seconds). Three replicates were performed and pooled. The pooled products were phenol extracted and precipitated as before and then re-suspended in 35 μl sterile distilled water.

Digestion to remove adaptors. The re-suspended DNA was then digested with 1 unit *Mlu*I (Amersham Pharmacia Biotech) with 2× reaction buffer in a total volume of 75 μl

at 37°C for 2 h. A clean up was performed using a Pharmacia S-300 spin column following the manufacturers instructions. 2× volume 1:1 phenol:chloroform was added and the mixture mixed and spun at 16000 rpm for 10 min. NaCl was added to 100 mM and the DNA precipitated in an equal volume of ethanol. The pellet was dried and re-suspended in 25 μl of sterile distilled water.

Plasmid preparation. A pJV1 plasmid was used in the ligations (this is a pUC19 plasmid containing a *BssH*II restriction site, modified and supplied by K. Edwards, IACR, Long Ashton, UK). Plasmid DNA was prepared from a 100 ml overnight culture using Quiagen maxi prep kit and 'Tip-500'. The prep was quantified on 1% agarose gel against standard quantities of λDNA..

100 μg of plasmid was digested with 50 units *Bss*HII (Amersham Pharmacia Biotech) and 1× reaction buffer in 300 μl at 50°C for 3 h. 100 μl of the digest was then dephosphorylated with 2 μl CIAP and 11 μl accompanying buffer at 37°C for 30 min. A further 2 μl CIAP was added and incubated at 37°C for a further 30 min. A 1:1 phenol:chloroform cleanup and ethanol precipitation was performed as before. The pellet was resuspended in 300 μl and quantified on 1% agarose gel.

Ligation into the plasmid vector. The ligation reaction was set up on ice. The inclusion of *Mlu*I in the ligation ensures that each plasmid contains only a single insert. 2 μl of the enriched, digested DNA (library) was ligated at 16°C for 4 h with 50 ng digested dephosphorylated plasmid, 1× reaction buffer, 2 μl 10 mM ATP, 0.5 units T4 DNA ligase (Amersham Pharmacia Biotech) and 0.15 units *Mlu*I (Amersham Pharmacia Biotech), in a total volume of 21 μl. This was followed by 10 min incubation at 65°C to stop the reaction.

The inserts had now been ligated into the plasmid and were ready for transforming into *Escherichia coli* cells.

Section 2) Pre-sequencing duplication of microsatellites

Preparation of *Escherichia coli* XL1 competent cells. *E. coli* XL1 cells (Gibco BRL, Cambridge, UK) were inoculated into 10 ml sterile Luria Broth (LB) (1% Bacto

Tryptone, 0.5% Bacto Yeast Extract, 1.0% NaCl) with 10 μl 10 mg/ml sterile tetracycline. The culture was grown at 37°C overnight.

5 ml of overnight culture was inoculated into each of two sterile flasks containing 500 ml sterile LB with 10 mg/ml tetracycline and incubated at 37°C with shaking at 250 rpm until an absorbance at OD 600 nm of 0.6 was reached. Cultures were then chilled on ice for 30 min and centrifuged in 165 ml aliquots at 4000 rpm at 4°C for 10 min and drained. The re-suspension and centrifugation procedure was repeated several times, resuspending the pellets in 160 ml ice cold sterile distilled water, then 80ml ice cold sterile distilled water, then 3 ml ice cold sterile 10% glycerol and finally 0.5 ml ice cold sterile 10% glycerol. The final suspensions were stored in 80 μl aliquots at -80°C.

Electrotransformation of *E. coli* XL1 competent cells. 4 μl ice cold sterile distilled water and 1 μl ice cold ligation mix (the ligated plasmid containing the library insert) was electro-transformed into 40 μl chilled competent cells (Celljject basic, Flowgen, Ashby de la Zouch, UK). 500 μl of sterile SOC (2% Bacto Tryptone, 0.5% Bacto Yeast Extract, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$, 20 mM glucose) was added immediately. Transformed bacteria were incubated at 37°C for 10 min and then plated onto LB-agar plates (LB + 1.5% agar) containing 150 μg/ml ampicillin, 40 μg/ml IPTG and 40 μg/ul Xgal. Following an overnight incubation at 37°C and then 4 h at 4°C, positive colonies (those that appear white rather than blue) were transferred into microtitre plates of sterile LB-glycerol (1% Bacto Tryptone, 0.5% Bacto Yeast Extract, 1.0% NaCl, 20% glycerol), containing 150 μg/ml ampicillin. Microtitre plates were incubated at 37°C overnight and then stored at -80°C.

Section 3) Detection and characterisation of microsatellites

10cm × 10cm pieces of Hybond membrane were placed on sterile LB-agar plates containing 150 μg/ml ampicillin, 40 μg/ml IPTG and 40 μg/ul Xgal. Colonies were transferred from previously prepared microtitre plates to the Hybond membrane filters using a sterile 'hedgehog' (an aluminium block with 8 ×12 pins that correspond to the positions of the microtitre plate wells). The plates were incubated at 37°C overnight.

The filters were then placed on two sheets of Whatman 3MM filter paper (Whatman plc, Maidstone, UK) soaked with 2× SSC/5% SDS at 60°C. 2× SSC/5% SDS was then gently pipetted over the bacterial colonies and the Hybond filter and filter paper drained and microwaved on full power until crisp. The filters were then stored at room temperature until ready for probing.

Filters were rinsed twice in 2× SSC. Up to 10 filters were incubated for 1 hour at 65°C with gentle shaking in 100ml prehybridisation solution (0.25 M $NaHPO_4$, 7% SDS, 1 mM EDTA) with 1% bovine serum albumin (BSA) fraction V. A blank piece of Hybond membrane was included as a negative control. Hybond pieces spotted with the probes to be used (at 50 ng/mm^2) denatured by boiling and UV fixed, served as positive controls.

The following probes were used: $[GC]_{17}$, $[AC]_{17}$, $[GA]_{17}$, $[GT]_{17}$,$[CAA]_{10}$, $[GCC]_{10}$, $[CTG]_{10}$, $[CAG]_{10}$

50 ng of each probe was boiled for 10 min in 30.5 μl of sterile distilled water, then immediately placed on ice. 6.0 μl OLB (a 10:25:15 mix of solutions A:B:C; solution A: 1.25 M Tris HCl, 0.125 M $MgCl_2$, 14.3 M β mercaptoethanol and 0.1 M each dATP, dTTP, dGTP; solution B: 2 M HEPES pH 6.6; solution C: 1 M Tris pH 7, 0.25 M EDTA, Hexanucleotides at 90 OD units/ml) and 1.2 μl BSA (10 mg/ml) was added to each probe.

0.5 μl ^{32}P-dCTP (370 MBq ml, 111 TBq mmol) and 3.6 units Klenow (Amersham Pharmacia Biotech) was then added to each probe and the probes incubated at 37°C for 1 h. 200 μl sterile distilled water was then added and probes boiled for 10 min. Probes were then added to the prehybridisation solution containing the Hybaid filters and incubated at 65°C with gentle shaking overnight.

Following incubation, filters were washed twice for fifteen minutes in 0.1× SSC/0.1% SDS with gentle shaking at 65°C. 10 minute washes in 2× SSC/0.1% SDS were then repeated as above until the radioactivity of the filters fell between 20 and 50 cps and the control (blank Hybond membrane) was near background level. Finally, filters were washed in 2× SSC for 10 min.

Filters were blotted between pieces of Whatman 3MM filter paper, sealed in cling film and placed with X-ray film in X-ray cassettes at -70°C for two days. Films were then developed using an automatic developer (Optimax, IGP, Chelmsford, UK). The co-ordinates of positive colonies (which appear as large dark spots on the X ray film) were then compared against their position on the filters to locate the corresponding clones containing microsatellites in the microtitre plate from which the filter was prepared.

DNA sequencing. Positive colonies were inoculated into 5 ml sterile LB containing 150 μg/ml ampicillin and incubated overnight. Cultures were centrifuged at 4000 rpm for 10 min and the pellets placed on dry ice. Minipreping from bacterial pellets and sequencing reactions were carried out by J. Masters at the Protein and Nucleic Acid Laboratory (PNACL, University of Leicester, UK), using NC45 (forward) and NC46 (reverse) primers (NC45: CAGCTATGACCATGATTACG; NC46: ACGTTGTAAAACGACGGCCAG; Edwards *et al.* 1996).

Section 4) Sequence comparison and primer design

DNA sequences were analysed and edited using SeqEd v1.0.3 (Applied Biosystems, Foster City, CA, USA). Vector sequence was deleted leaving the *Cirsium acaule* genomic DNA insert. Forward and reverse sequences were aligned and a consensus sequence created. All sequences were then compared using Gene Jockey (1990, Biosoft, Cambridge, UK) to ensure they were unique. Primers were then designed using Primer 3 (Rozen & Skaletsky 1996,1997).

Primer screening.

Primers developed to amplify microsatellites in *Cirsium acaule* were tested on DNA from five individuals of each of *C. acaule*, *C. eriophorum*, *C. arvense* and *C. heterophyllum*. Individuals used for the primer screening were selected at random from throughout the geographic range of these species.

PCR reactions were carried out in 10 μl volumes containing 50 ng genomic DNA, 1 μM of each forward and reverse primer, 0.25 units Thermoprime Plus DNA polymerase

(ABGene, Epsom, Surrey, UK) in the manufacturer's buffer (final concentrations 20 mM $(NH_4)_2SO_4$, 75 mM Tris-HCl pH9.0, 0.01% Tween) and 1.5-2.5 mM $MgCl_2$ (see Table 5.2.2), 200 μM of each dNTP with the addition of 0.5% Tween 20 (final concentration). PCR amplification was performed in a Hybaid Touchdown thermal cycler (Thermo Hybaid, Ashford, Middlesex, UK).

A touchdown PCR programme was used: Stage 1: 94°C/3 min, followed by stage 2 consisting of 2 cycles of 94°C/30s 65°C/30s 72°C/30s. Subsequent stages then reduced annealing temperature in steps of 1°C from 65°C to 57°C, at each stage 2 cycles were performed as above. The programme then continued with 94°C/30s 56°C/30s 72°C/30s for 15 cycles before a final 3min 72°C extension stage.

5 μl stop (10 ml formamide, 10 mg xylene cyanol, 10 mg bromophenol blue added to 200 μl 0.5 M EDTA) was added to PCR products and the mixture denatured at 95°C for five minutes, a fifty base pair size marker was also denatured in this way. Denatured products were immediately placed on ice. 3.5 μl of the denatured product was electrophoresed in one lane of a 6% polyacrylamide gel at 70 W constant wattage using 1× TBE running buffer. DNA was then visualised using the silver staining technique (Bassam *et al.* 1991).

Primer screening suggested that few of the *Cirsium acaule* primers would amplify polymorphic loci reliably in *C. eriophorum*. *C. eriophorum* occurs in a different subsection of the genus to the other *Cirsium* species tested here (Tutin *et al.* 1976) which may partly explain the relatively poor amplification in this species. When combined with time constraints, this led to *C. eriophorum* not being included in the molecular genetic analysis. Polymorphic loci selected for genotyping of *C. acaule*, *C. arvense* and *C. heterophyllum* are listed in Table 5.2.1.

Table 5.2.1 Polymorphic microsatellite loci used to genotype *Cirsium* species

Species	Loci
Cirsium acaule	*Caca01 Caca04 Caca05 Caca07 Caca16 Caca24*
Cirsium arvense	*Caca01 Caca04 Caca05 Caca10*
Cirsium heterophyllum	*Caca01 Caca04 Caca10 Caca16 Caca17 Caca22 Caca24*

Following the identification of polymorphic loci, it was necessary to determine which primer of each pair should be labelled with a fluorescent phosphoramidite (TET, HEX or FAM, Applied Biosystems) in order to facilitate semi-automated analysis of products using an ABI 377 DNA sequencer (Applied Biosystems).

Reaction sets were set up with primer pairs designed to amplify each locus listed in Table 5.2.1. DNA from one individual of *C. acaule* was amplified under the conditions listed above. Each set consisted of a reaction using only forward primer (at 2 μM), one using only reverse primer (at 2 μM) and one using both forward and reverse primers (1 μM each). Products were visualised using 6% polyacrylamide gel and silver staining as before.

Of the forward + forward and reverse + reverse reactions, those giving no product or the least defined product were chosen to be flourescently labled. Where no product was seen with either combination, the forward primer was labled by default. The sequence: 5'GTTTCTT3' was added to the 5' end of the unlabled primer from each pair to reduce genotyping error due to variable adenylation during PCR. (Brownstein *et al.* 1996). Primer sequences and 5' modifications are given in Table 5.2.2. A full description of the characteristics of these loci can be found in Jump *et al.* (2002), Appendix B.

5.2.4 Amplification of microsatellites from genomic DNA

Microsatellite loci listed in Table 5.2.1 were amplified from 25 individuals from each of the populations detailed in Table 1.3.1; for *C. heterophyllum*, this included four additional populations from the Swiss and Italian Alps not included in the productivity and seed production surveys presented in Chapter 4. PCR conditions are listed in Table 5.2.2.

For the genotyping of individuals, DNA was not purified. DNA fragments containing microsatellites were amplified using PCR from crude leaf extracts prepared according to a protocol based on that presented by Wang *et al.* (1993) and tested by Rogers *et al.* (1996). BSA (fraction V) was added to PCR conditions after Möhlenhoff *et al.* (2001).

Table 5.2.2 Amplification and polymorphism of *Cirsium acaule* microsatellite loci in *C. acaule, C. arvense* and *C. heterophyllum* ($N = 25$).

Locus	Modification at 5′ primer end (5′-3′)	Primer sequence (5′-3′)	Cycling conditions		Number of alleles		
			Program	$MgCl_2$ (mM)	*Cirsium acaule*	*Cirsium arvense*	*Cirsium heterophyllum*
Caca01	F: HEX-	TTT GAA GTG GAT CTT CGC ACG	64	2.5	4	5	2
	R: GTTTCTT-	CAT GGG AGA CGA ACT AAC AGA TGC					
Caca04	F: GTTTCTT-	ATC ACC GCT TCC ACC GTC TC	65/55TD	2.5	3	5	3
	R: FAM-	GCT TAT TAG AAC CGC CAT TGA AAG C					
Caca05	F: TET-	ACC CAA CCC TCG ATC TGA A	62/52TD	1.5	2	7	1
	R: TTTCTT-	GAG GAT ACC GGC GAT TGT TA					
Caca07	F: GTTTCTT-	CCC AAA CTC CCA CCT TCA TTT G	64	2.5	5	NCA (7)	NP
	R: HEX-	GTC GGA GAT GCT CCG GTG AC					
Caca10	F: GTTTCTT-	GAA TTC GCG ACA ACA CAC GC	65/55TD	1.5	NCA (4)	6	2
	R: FAM-	GGT AAG GAA TGA ATG ATT GGG CTC					
Caca16	F: TET-	TCG TGC TCT TCG ATT GAT TG	60	2.5	4	NP	3
	R: GTTTCTT-	CAG AAA ACC GCT CCA TTG C					
Caca17	F: TET-	GGC ATA CTG ACA TTC TCA AAC GC	55	1.5	NCA (2)	1	3
	R: GTTTCTT-	CGT GAT GTG ATG GCA TGT TC					
Caca22	F: TET-	GGC TCT GCC TCA CCC ATC TC	65/55TD	1.5	1	NCA (7)	3
	R: GTTTCTT-	AGG TGT TCA GCA CGG TTC GG					
Caca24	F: HEX-	TGG ATA ACG CGC TAG ATC AC	62/52TD	2.5	7	1	4
	R: GTTTCTT-	AAG AAC TCA ATT AGT AGG AAG TGG					

PCR Programs

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)

Note: NP = no product of the expected size, NCA = not consistently amplifying across all individuals.

Preparation of leaf extract. Approximately 0.5 cm^2 silica gel dried leaf tissue was ground in a 1.5 ml sterile tube in 60 μl 0.5 M NaOH. A sterile micropestle fitted to a cordless hand drill and used on a low speed, together with the addition of a small pinch of sterile sand aided grinding. When no large leaf fragments remained, the suspension was centrifuged at 14000 rpm for five min. 15 μl of the supernatant was then added to 485 μl sterile 100 mM Tris-HCl (pH8) and mixed well. 2 μl of this extract was then used directly in each PCR reaction

2 μl template DNA was amplified in a total volume of 15 μl containing 3 mg/ml BSA (fraction V), 0.5% Dimethylsulfoxide (DMSO), 1× PCR buffer, 1.5 mM $MgCl_2$, 200 μM each dATP, dCTP, dGTP,and dTTP, 1μM each forward and reverse primer, 0.25 units Thermoprime Plus DNA polymerase (ABGene), 1.5/2.5 mM $MgCl_2$ (Table 5.2.2). PCR was performed in a Hybaid Touchdown Thermal Cycler according to the programs detailed in Table 5.2.2.

Products were analysed on 5% polyacrylamide gels using an ABI 377 Sequencer running GENESCAN v3.1.2 software (Applied Biosystems). Genotypes were assigned using GENOTYPER v2.5 (Applied Biosystems).

5.2.5 Data analysis

For *Cirsium arvense* and *C. heterophyllum*, statistics were calculated twice: 1) using each sampled plant (ramet level analysis) and 2) after the removal of duplicate multilocus genotypes from within each population (genet level analysis). These species were sampled within dense stands and thus individual genets could not be identified at the time of sampling. If duplicate multilocus genotypes are not removed, then a single genetic individual may be represented several times in the same data set. An intact data set could be biased because samples are not independent. However, removing duplicate multilocus genotypes may result in the over-representation of rare alleles and the under representation of common alleles. Calculating statistics based for both the ramet and genet data set will indicate both the range of possible genetic diversity values for the species and the effects of clonal reproduction on diversity and population structure (McClintock and Waterway 1993; Ivey and Richards 2001). Duplicate multilocus

genotypes were extremely rare within samples representing populations of *C. acaule* as in this species plants grow as distinct patches (presumed genets) and only one sample was taken from any one patch within a population.

Observed heterozygosity (H_o) and expected heterozygosity (H_e) were calculated using GENETIX v4.02 (Belkhir *et al.* 2001). FSTAT v2.9.3.2 2002 (Goudet 1995) was used to calculate allelic richness and Nei's gene diversity statistics (Nei 1987). FSTAT was also used to test for deviation from Hardy-Weinberg equilibrium (HWE) within populations as well as for deviation from HWE for each polymorphic locus within populations. These tests were based on permutations of the data, in which alleles were randomised within populations (*C. acaule*: 1,680 permutations; *C. arvense*: 1,280; *C. heterophyllum*: 3,220). Loci were considered to be in HWE if greater than 5% of randomised data sets resulted in fixation indices (F_{IS}; Weir and Cockerham 1984) that were more extreme than those observed. Because the *C. arvense* genet data set contained some populations with an extremely small sample size only populations with at least four genets were included for calculation of Nei's gene diversity statistics (after McClintock and Waterway 1993). This resulted in the exclusion of populations RD3, RS2 and RN1 from this analysis.

Population differentiation over all populations was assessed based on randomising genotypes among populations (not assuming HWE) and the log-likelihood statistic *G* (Goudet *et al.* 1996) calculated in FSTAT. Significance levels were adjusted by sequential Bonferroni corrections (Rice 1989). One thousand randomisations were performed for each data set.

Clonal diversity analysis. In clonal species the number and relative frequency of multilocus genotypes are important measures of genetic diversity (Ellstrand and Roose 1987). For *Cirsium arvense* and *C. heterophyllum*, mean clone size was calculated by dividing the number of shoots sampled by the number of clones found. The Simpson diversity index (D) modified for finite samples (Pielou 1969) was calculated for each population, $D = 1 - \Sigma[N_j(N_j - 1)/N(N - 1)]$, where N_j is the number of shoots of the *j*th genotype and N is the sample size. This measure was originally devised as a measure of species diversity but has been applied to measure the diversity of clones within a population (McClintock and Waterway 1993, Vasseur 2001). Fager's (1972) E was

also calculated, $E = (D - D_{min})/(D_{max} - D_{min})$. E describes the evenness of the distribution of genotypes within the population, Like D it varies between 0 and 1.

To assess whether diversity was related to population size, the Pearson product-moment correlation coefficient was calculated between population area (the surrogate for population size) and all diversity measures using Minitab v11.2 (Minitab inc., State College, PA, USA).

To estimate genetic divergence among populations within each species, Nei's (1972) genetic distance was calculated for all possible pairs of populations using *Gendist.* Un-rooted trees representing (Nei's 1972) genetic distance were created in *Neighbour* using the unweighted pair group method (UPGMA) of clustering, these were then plotted using *Drawtree.* Gendist, Neighbour and Drawtree are part of the PHYLIP v3.5c group of programmes (Felsenstein 1989).

Genetic isolation by geographic distance. Within each species, geographic distance between all possible pairs of populations was calculated from site latitude and longitude (Table 1.3.1) using a FORTRAN 77 programme written by M.R. Lomas of the University of Sheffield, UK. Genetic isolation by distance was assessed using the programme IBD v1.2 (Bohonak 2002). For *C. heterophyllum* this analysis was repeated following the removal of all non-UK populations from the data set. To overcome problems of non-independence in repeated comparison of data points, a Mantel test was performed using IBD on the correlation between geographic distance and genetic distance, based on 10,000 randomisations of the data. The slope and intercept of any relationship were calculated in IBD by Reduced Major Axes regression (RMA) and confidence limits calculated based on bootstrap resampling of the data sets. Confidence limits were based on 10,000 bootstrap re-samples of the data.

5.3.1 Results

Genetic diversity within populations

Only *Cirsium acaule* shows a significant relationship between genetic diversity and latitude. In *C. acaule*, allelic richness (a measure of the number of alleles independent of sample size) decreases with increasing latitude ($R^2 = 0.55$, $P < 0.005$, Fig. 5.1.1). Maximum allelic richness in *C. acaule* was found to be 5.43 in Wiltshire in the core area of its UK distribution declining to a maximum of 3.00 in the peripheral, Peak District populations.

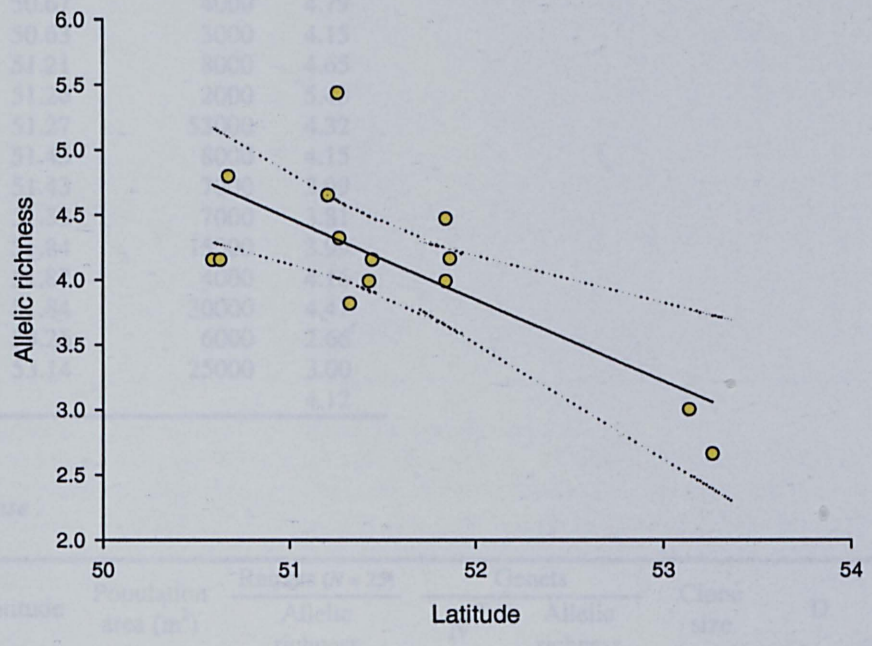

Fig. 5.1.1 Mean allelic richness (averaged over loci) in *C. acaule* populations as a function of latitude. Regression: $y = 36.36 - 0.63x$, $R^2 = 0.55$, $P < 0.005$. Dotted lines show 95% confidence interval of regression.

No relationship between genetic diversity and latitude is seen in either *C. arvense* or *C. heterophyllum* at either the ramet or the genet level. Allelic richness is a measure of the number of alleles present, adjusted for by the sample size. Allelic richness is generally lower when populations were analysed at the genet level rather than the ramet level. This is likely to be an artifact of the reduction in sample size that results from removing duplicate multilocus genotypes (ramets) from the within each population, since this process does not lead to the complete removal of alleles from the population as the genet (of which ramets are duplicates) still remains. There is no relationship with

latitude in clonal diversity (D), evenness (E), or clone size in either *C. arvense* or *C. heterophyllum* (Table 5.3.1b,c).

There was no significant correlation between any measure of genetic diversity and population area in any species.

Table 5.3.1 Genetic diversity in *Cirsium* populations.

a) *C. acaule*

Pop	Latitude	Population area (m^2)	Allelic richness
AD1	50.59	73000	4.15
AD2	50.67	4000	4.79
AD3	50.63	3000	4.15
AW1	51.21	8000	4.65
AW2	51.26	2000	5.43
AW3	51.27	53000	4.32
AB1	51.45	8000	4.15
AB2	51.43	7000	3.99
AB3	51.33	7000	3.81
AG1	51.84	15000	3.99
AG2	51.87	4000	4.16
AG3	51.84	30000	4.47
AP1	53.26	6000	2.66
AP2	53.14	25000	3.00
Mean			4.12

b) *C. arvense*

Pop	Latitude	Population area (m^2)	Ramets ($N = 25$)	Genets		Clone size	D	E
			Allelic richness	N	Allelic richness			
RD2	50.68	750	4.98	12	2.71	2.1	0.89	0.90
RD3	50.59	480	1.75	2	1.75	12.5	0.08	0.00
RB1	51.44	1400	4.72	18	2.40	1.4	0.97	1.00
RB2	51.43	3000	4.49	8	2.61	3.1	0.75	0.75
RG1	51.84	1300	5.46	14	2.39	1.8	0.92	0.94
RG2	51.86	160	5.44	9	2.66	2.8	0.71	0.70
RP1	53.21	210	5.97	13	2.80	1.9	0.91	0.93
RP2	53.14	310	5.93	16	2.64	1.6	0.92	0.94
RC2	54.53	90	5.22	14	2.57	1.8	0.92	0.94
RC3	54.53	120	3.74	8	2.44	3.1	0.80	0.80
RS1	56.39	15	3.43	8	2.03	3.1	0.49	0.46
RS2	56.36	40	2.21	3	1.83	8.3	0.16	0.09
RM1	57.10	225	5.25	11	2.77	2.3	0.93	0.95
RM2	57.00	180	3.73	14	2.23	1.8	0.92	0.94
RN1	57.97	36	2.00	2	2.00	12.5	0.08	0.00
RN2	58.16	200	2.74	8	2.15	3.1	0.49	0.46
Mean			4.19	10	2.37	4.0	0.68	0.68

Table 5.3.1 (continued)

c) *C. heterophyllum*

Pop	Latitude	Population area (m^2)	Ramets ($N = 25$)	Genets		Clone size	D	E
			Allelic richness	N	Allelic richness			
HA1	46.10	35	2.72	21	2.33	1.2	0.98	0.98
HA2	45.84	45	2.24	18	2.01	1.4	0.94	0.91
HA3	45.84	2150	2.57	21	2.10	1.2	0.98	0.98
HA4	45.94	1850	2.78	13	2.41	1.9	0.80	0.67
HP1	53.21	45	2.32	18	2.13	1.4	0.98	0.97
HP2	53.23	30	2.01	10	2.02	2.5	0.75	0.57
HP3	53.24	100	1.57	4	1.57	6.3	0.42	0.00
HP4	53.17	860	2.41	16	2.21	1.6	0.94	0.90
HC1	54.41	72	2.39	11	2.25	2.3	0.88	0.80
HC2	54.44	120	3.19	15	2.67	1.7	0.87	0.78
HC3	54.86	100	2.89	19	2.41	1.3	0.97	0.96
HC4	54.45	900	3.15	24	2.60	1.0	1.00	1.00
HC5	54.38	18	1.81	7	1.87	3.6	0.59	0.29
HS1	56.49	200	2.61	16	2.27	1.6	0.95	0.91
HS2	56.40	30	1.88	10	1.89	2.5	0.78	0.63
HS3	56.32	160	2.32	15	2.12	1.7	0.93	0.89
HM1	57.10	150	2.98	21	2.55	1.2	0.98	0.97
HM2	57.01	340	3.30	21	2.71	1.2	0.97	0.95
HM3	57.33	40	2.02	7	2.07	3.6	0.59	0.29
HM4	57.42	50	2.69	19	2.27	1.3	0.97	0.96
HN1	57.99	40	2.33	17	2.16	1.5	0.95	0.93
HN2	58.24	70	2.58	19	2.39	1.3	0.97	0.95
HN3	57.75	30	2.15	12	2.05	2.1	0.88	0.79
Mean			2.47	15	2.22	2.0	0.87	0.79

Notes:
Allelic richness indicates mean allelic richness averaged over loci.
In *C. arvense* and *C. heterophyllum,* results are presented for all sampled shoots (ramets) and after removal of duplicate genotypes from within populations (genets). In genet level analysis: N = the number of individuals in each population after duplicate multilocus genotypes have been removed. Clone size = the number of shoots per clone (N ramet/N genet). D = Simpson's diversity index. E = Fager's measure of sample evenness.

Population genetic structure

In *Cirsium acaule,* all populations with the exception of population AG1 were found to be in Hardy Weinberg equilibrium (HWE). A significant excess of heterozygotes (negative F_{IS}) was observed in AG1 ($P < 0.05$, Table 5.3.2a).

In *C. arvense,* 69% of populations showed an excess of heterozygotes when analysed at the ramet level. At the genet level, 56% of populations of *C. arvense* show an excess of heterozygotes whereas 13% show an excess of homozygotes (positive F_{IS}; Table 5.3.2b).

In *C. heterophyllum* 52% of populations showed an excess of heterozygotes and 17% an excess of homozygotes when analysed at the ramet level. At the genet level, 48% of populations show an excess of heterozygotes whereas 13% show an excess of homozygotes (Table 5.3.2c).

Table 5.3.2 Mean (SE) observed (H_o) and expected (H_e) heterozygosity across loci within populations and within population estimates of inbreeding (F_{IS}) for *Cirsium* species

a) *C. acaule*

Pop	H_o	*SE*	H_e	*SE*	F_{IS}
AD1	**0.598**	*0.043*	**0.586**	*0.050*	0.000
AD2	**0.620**	*0.042*	**0.572**	*0.054*	-0.064
AD3	**0.627**	*0.042*	**0.610**	*0.039*	-0.008
AW1	**0.620**	*0.052*	**0.613**	*0.043*	0.010
AW2	**0.667**	*0.038*	**0.630**	*0.044*	-0.038
AW3	**0.628**	*0.056*	**0.618**	*0.053*	-0.033
AB1	**0.633**	*0.068*	**0.637**	*0.053*	0.026
AB2	**0.613**	*0.086*	**0.613**	*0.069*	0.020
AB3	**0.573**	*0.075*	**0.538**	*0.064*	-0.045
AG1	**0.680**	*0.045*	**0.606**	*0.043*	-0.102*
AG2	**0.593**	*0.069*	**0.618**	*0.043*	0.060
AG3	**0.673**	*0.060*	**0.615**	*0.061*	-0.075
AP1	**0.513**	*0.041*	**0.499**	*0.040*	-0.008
AP2	**0.500**	*0.068*	**0.505**	*0.072*	0.030

Note. All P values were obtained in a randomisation test of $F_{IS} = 0$ based on 1680 permutations of the data in which alleles were randomised within populations. * $P < 0.05$

b) *C. arvense*

Pop	*Ramets*					*Genets*				
	H_o	*SE*	H_e	*SE*	F_{IS}	H_o	*SE*	H_e	*SE*	F_{IS}
RD2	**0.740**	*0.096*	**0.709**	*0.043*	-0.023	**0.688**	*0.120*	**0.702**	*0.041*	0.064
RD3	**0.740**	*0.247*	**0.375**	*0.125*	-0.973***	**0.625**	*0.239*	**0.344**	*0.118*	-0.667***
RB1	**0.660**	*0.159*	**0.619**	*0.049*	-0.046	**0.667**	*0.164*	**0.619**	*0.051*	-0.048
RB2	**0.680**	*0.217*	**0.556**	*0.168*	-0.204**	**0.750**	*0.177*	**0.623**	*0.137*	-0.139*
RG1	**0.540**	*0.213*	**0.545**	*0.164*	0.029	**0.518**	*0.199*	**0.563**	*0.131*	0.116*
RG2	**0.650**	*0.205*	**0.552**	*0.147*	-0.158**	**0.583**	*0.172*	**0.642**	*0.120*	0.149*
RP1	**0.690**	*0.145*	**0.674**	*0.102*	-0.003	**0.673**	*0.110*	**0.715**	*0.065*	0.098
RP2	**0.740**	*0.118*	**0.636**	*0.037*	-0.144*	**0.766**	*0.100*	**0.685**	*0.033*	-0.086
RC2	**0.640**	*0.108*	**0.605**	*0.083*	-0.037	**0.677**	*0.084*	**0.654**	*0.078*	0.002
RC3	**0.660**	*0.185*	**0.500**	*0.118*	-0.302***	**0.750**	*0.125*	**0.607**	*0.074*	-0.171*
RS1	**0.540**	*0.254*	**0.335**	*0.107*	-0.600***	**0.625**	*0.184*	**0.481**	*0.047*	-0.239*
RS2	**0.270**	*0.230*	**0.159**	*0.120*	-0.690***	**0.417**	*0.083*	**0.333**	*0.056*	-0.053***
RM1	**0.920**	*0.033*	**0.720**	*0.020*	-0.259***	**0.886**	*0.044*	**0.712**	*0.023*	-0.200**
RM2	**0.820**	*0.062*	**0.565**	*0.022*	-0.436***	**0.786**	*0.077*	**0.572**	*0.023*	-0.341***
RN1	**0.990**	*0.010*	**0.500**	*0.000*	-0.980***	**0.875**	*0.125*	**0.469**	*0.031*	-0.750***
RN2	**0.750**	*0.224*	**0.454**	*0.045*	-0.639***	**0.750**	*0.169*	**0.551**	*0.056*	-0.302*

Note. All P values were obtained in a randomisation test of $F_{IS} = 0$ based on 1220 permutations of the data in which alleles were randomised within populations. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Analysis of all sampled shoots (ramets) and after removal of duplicate genotypes within populations (genets).

Table 5.3.2. (continued)

c) *C. heterophyllum*

Pop	*Ramets*					*Genets*				
	H_o	*SE*	H_e	*SE*	F_{IS}	H_o	*SE*	H_e	*SE*	F_{IS}
HA1	**0.497**	*0.094*	**0.440**	*0.063*	-0.111*	**0.490**	*0.137*	**0.443**	*0.083*	-0.082
HA2	**0.320**	*0.183*	**0.311**	*0.089*	-0.007	**0.310**	*0.099*	**0.342**	*0.071*	0.126
HA3	**0.303**	*0.108*	**0.342**	*0.030*	0.135**	**0.327**	*0.091*	**0.356**	*0.087*	0.107
HA4	**0.366**	*0.135*	**0.370**	*0.072*	0.032	**0.417**	*0.121*	**0.418**	*0.095*	0.047
HP1	**0.543**	*0.127*	**0.436**	*0.079*	-0.225***	**0.549**	*0.125*	**0.446**	*0.029*	-0.204**
HP2	**0.634**	*0.158*	**0.371**	*0.083*	-0.697***	**0.587**	*0.116*	**0.393**	*0.056*	-0.447***
HP3	**0.537**	*0.173*	**0.286**	*0.062*	-0.877***	**0.464**	*0.176*	**0.281**	*0.100*	-0.560**
HP4	**0.577**	*0.122*	**0.433**	*0.060*	-0.315***	**0.571**	*0.135*	**0.454**	*0.048*	-0.228**
HC1	**0.394**	*0.121*	**0.367**	*0.071*	-0.055	**0.429**	*0.128*	**0.399**	*0.088*	-0.026
HC2	**0.429**	*0.135*	**0.411**	*0.095*	-0.023	**0.448**	*0.105*	**0.480**	*0.062*	0.102
HC3	**0.359**	*0.095*	**0.431**	*0.058*	0.188***	**0.344**	*0.105*	**0.438**	*0.069*	0.243***
HC4	**0.463**	*0.193*	**0.526**	*0.096*	0.141***	**0.451**	*0.096*	**0.529**	*0.057*	0.170**
HC5	**0.457**	*0.128*	**0.283**	*0.022*	-0.602***	**0.510**	*0.181*	**0.331**	*0.106*	-0.485***
HS1	**0.417**	*0.043*	**0.388**	*0.058*	-0.054	**0.420**	*0.109*	**0.415**	*0.058*	0.020
HS2	**0.634**	*0.145*	**0.372**	*0.080*	-0.693***	**0.600**	*0.135*	**0.399**	*0.076*	-0.465***
HS3	**0.457**	*0.108*	**0.422**	*0.069*	-0.062	**0.419**	*0.113*	**0.427**	*0.078*	0.054
HM1	**0.526**	*0.134*	**0.497**	*0.096*	-0.038	**0.544**	*0.095*	**0.504**	*0.059*	-0.054
HM2	**0.339**	*0.137*	**0.475**	*0.081*	0.306***	**0.349**	*0.035*	**0.509**	*0.060*	0.336***
HM3	**0.611**	*0.088*	**0.340**	*0.087*	-0.792***	**0.653**	*0.173*	**0.389**	*0.106*	-0.634***
HM4	**0.640**	*0.143*	**0.427**	*0.053*	-0.483***	**0.639**	*0.131*	**0.444**	*0.075*	-0.418***
HN1	**0.686**	*0.109*	**0.447**	*0.076*	-0.521***	**0.689**	*0.138*	**0.455**	*0.085*	-0.493***
HN2	**0.707**	*0.192*	**0.517**	*0.101*	-0.348***	**0.701**	*0.104*	**0.527**	*0.030*	-0.307***
HN3	**0.591**	*0.150*	**0.380**	*0.070*	-0.536***	**0.607**	*0.159*	**0.420**	*0.048*	-0.395***

Note. All P values were obtained in a randomisation test of $F_{IS} = 0$ based on 1220 permutations of the data in which alleles were randomised within populations. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Analysis of all sampled shoots (ramets) and after removal of duplicate genotypes within populations (genets).

Diversity within *Cirsium* species

Nei's gene diversity statistics (Nei 1987) are used to show the degree to which genetic diversity is distributed within and between populations. Both total diversity (H_T) and the proportion of genetic diversity within populations (H_S) was high for all species. In *C. acaule* only about 7% of total diversity present was distributed among populations (as indicated by G_{ST}) whereas G_{ST} was between 25% (ramet) and 13% (genet) in *C. arvense* and between 36% (ramet) and 32% (genet) in *C. heterophyllum*. There was little difference between ramet level and genet level estimates of H_T. H_S was higher at the genet level estimate and G_{ST} lower, in both *C. arvense* and *C. heterophyllum*.

The similarity in H_T values for the three *Cirsium* species investigated here indicates comparable levels of genetic variability within these species (Table 5.3.3). Population differentiation is particularly high in *C. heterophyllum* (G_{ST} = 0.318 – 0.359). In *C. arvense* population differentiation is also high (G_{ST} = 0.131 – 0.246). The low G_{ST} in *C. acaule* (G_{ST} = 0.066) suggests populations of this species are somewhat less differentiated, although this level of differentiation is still considered moderate by Balloux and Lugon-Moulin (2002).

Tests of population differentiation were significant at all loci and overall for all species and at both the ramet level and genet level of analysis (log-likelihood G, P< 0.001).

Table 5.3.3 Genetic diversity per locus and over all loci in *Cirsium* species.

a) *C. acaule*

Locus	H_T	H_S	G_{ST}
Caca01	0.701	0.656	0.065
Caca04	0.568	0.499	0.122
Caca05	0.510	0.492	0.036
Caca07	0.619	0.590	0.047
Caca16	0.641	0.597	0.068
Caca24	0.816	0.769	0.058
Mean	0.643	0.600	0.066

b) C. arvense

Locus	Ramets			Genets		
	H_T	H_S	G_{ST}	H_T	H_S	G_{ST}
Caca01	0.722	0.533	0.261	0.735	0.610	0.170
Caca04	0.470	0.377	0.198	0.566	0.538	0.050
Caca05	0.825	0.619	0.250	0.853	0.733	0.141
Caca10	0.844	0.628	0.256	0.848	0.729	0.140
Mean	0.715	0.539	0.246	0.751	0.653	0.131

Note. For genet level analysis, only populations containing 4 genets or more are included.

Table 5.3.3 (continued)

c) *C. heterophyllum*

Locus	Ramets			Genets		
	H_T	H_S	G_{ST}	H_T	H_S	G_{ST}
Caca01	0.604	0.330	0.454	0.608	0.364	0.401
Caca04	0.638	0.391	0.387	0.673	0.495	0.265
Caca10	0.761	0.532	0.301	0.762	0.541	0.291
Caca16	0.723	0.499	0.310	0.719	0.512	0.288
Caca17	0.469	0.409	0.128	0.481	0.431	0.104
Caca22	0.496	0.269	0.458	0.505	0.298	0.410
Caca24	0.780	0.437	0.439	0.778	0.448	0.424
Mean	0.639	0.410	0.359	0.647	0.441	0.318

Note. H_T = Total gene diversity, H_S = gene diversity within populations, G_{ST} = among population differentiation. Estimates calculated according to Nei (1987). For *C. arvense* and *C. heterophyllum* statistics are based on all sampled shoots (ramets) and after removal of duplicate genotypes within populations (genets).

Genetic distance and geographic structure

Mean Nei's (1972) genetic distance among population pairs was 0.147 (range: 0.034 to 0.440) in *C. acaule*. In C *arvense*, ramets: 0.553 (0.121 to 1.458); genets 0.490 (0.107 to 1.264). Mean genetic distance between *C. heterophyllum* population pairs was ramets: 0.555 (0.070 to 1.460) and genets: 0.533 (0.089 to 1.390).

Un-rooted tree diagrams representing Nei's (1972) genetic distance in each species are shown in figures 5.2.1 to 5.2.3.

Cirsium acaule populations form a relatively tight cluster with only two populations identified as outliers (populations AP1 and AP2, Fig. 5.2.1). In *C. acaule* the outliers indicated by genetic distance represent those populations that are found at the edge of the species geographic range. The genetic distance between populations AP1 and AP2 is 0.271 (62% of the maximum genetic distance recorded for this species). The mean genetic distance between either of these populations and any of the populations in the core area of the species range is 0.297 (70%), this contrasts with a mean genetic distance of 0.088 (19%) between core populations. Peripheral populations in *C. acaule* are remote from both each other and from populations in the core area of the species UK geographic range.

Cirsium arvense does not reach a geographic limit within the UK. The clustering of populations is somewhat looser at the genet level when compared with the ramet level of analysis (Fig. 5.2.2). At the genet level of analysis the tree structure corresponds broadly with the geographic areas of the UK within which the populations were sampled. Outlying *C. arvense* populations include populations from different latitudes. Only in the genet level analysis are all populations at a latitude classed as outliers (populations RN1 and RN2); the genetic distance between these is 0.552 (44% of the maximum genetic distance recorded for this species). The mean genetic distance between either of these populations and any of the populations in other areas of the species UK range is 0.757 (60%), which contrasts with a mean genetic distance of 0.482 (38%) between the main group of *C. arvense* populations. At the genet level of analysis of *C. arvense*, populations RN1 and RN2 are relatively less divergent both from each other and other *C. arvense* populations when compared with the peripheral populations of *C. acaule* (Fig. 5.2.1).

In *C. heterophyllum*, little geographical structuring of the tree is seen. Populations from the Swiss and Italian Alps form a tight cluster within the tree but this pattern is not seen with populations surveyed within any other broad geographic area. Groups of populations within the *C. heterophyllum* trees usually include populations from across the species UK geographic range. At both the ramet and genet level of analysis, geographically peripheral populations are not confined to a single cluster and occur throughout the tree. The outlying populations suggested by the trees are not as distant as those suggested in either the *C. acaule* tree or the *C. arvense* tree and include populations from both the core and the periphery of the species UK geographic range (Fig. 5.2.3).

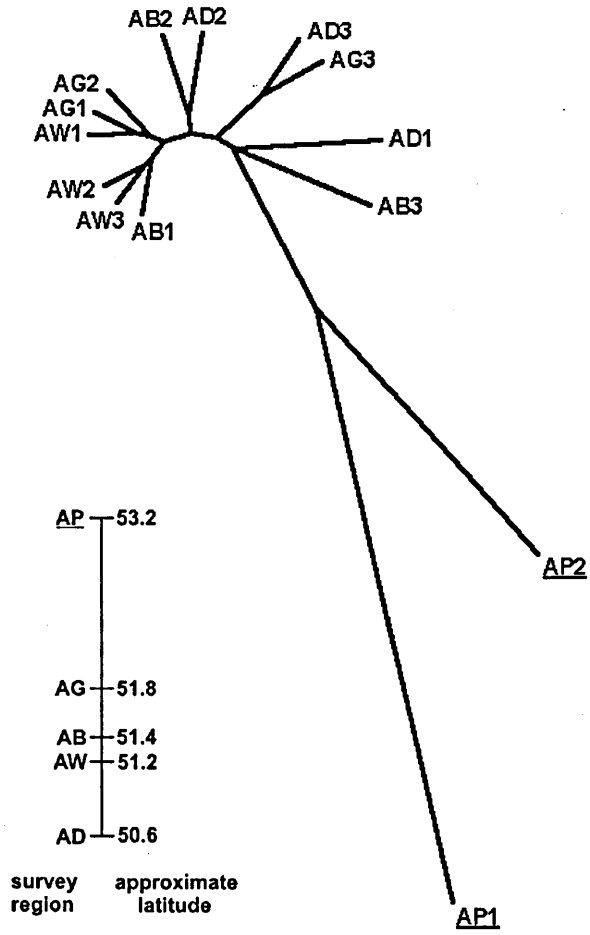

Fig. 5.2.1 Un-rooted tree from UPGMA cluster analysis based on Nei's (1972) genetic distance between *C. acaule* populations. Branch lengths are scaled relative to the maximum genetic distance between populations. Peripheral populations are underlined. Key shows approximate latitude of survey regions, accurate site locations are given in Table 1.3.1.

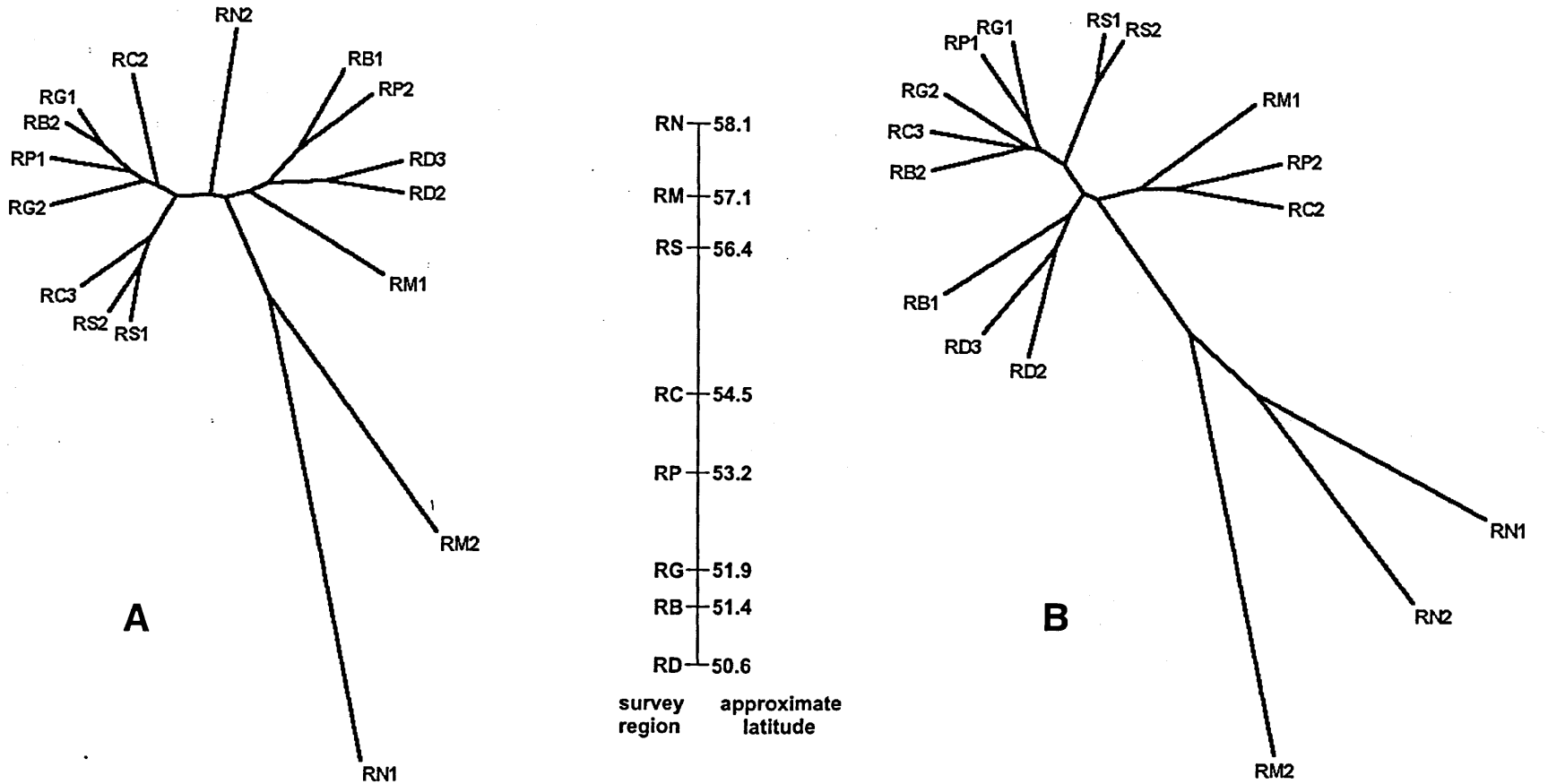

Fig. 5.2.2 Un-rooted tree from UPGMA cluster analysis based on Nei's (1972) genetic distance between *C. arvense* populations. Branch lengths are scaled relative to the maximum genetic distance between populations. Analyses are of ramets (A) and genets (B). Key shows approximate latitude of survey regions, accurate site locations are given in Table 1.3.1.

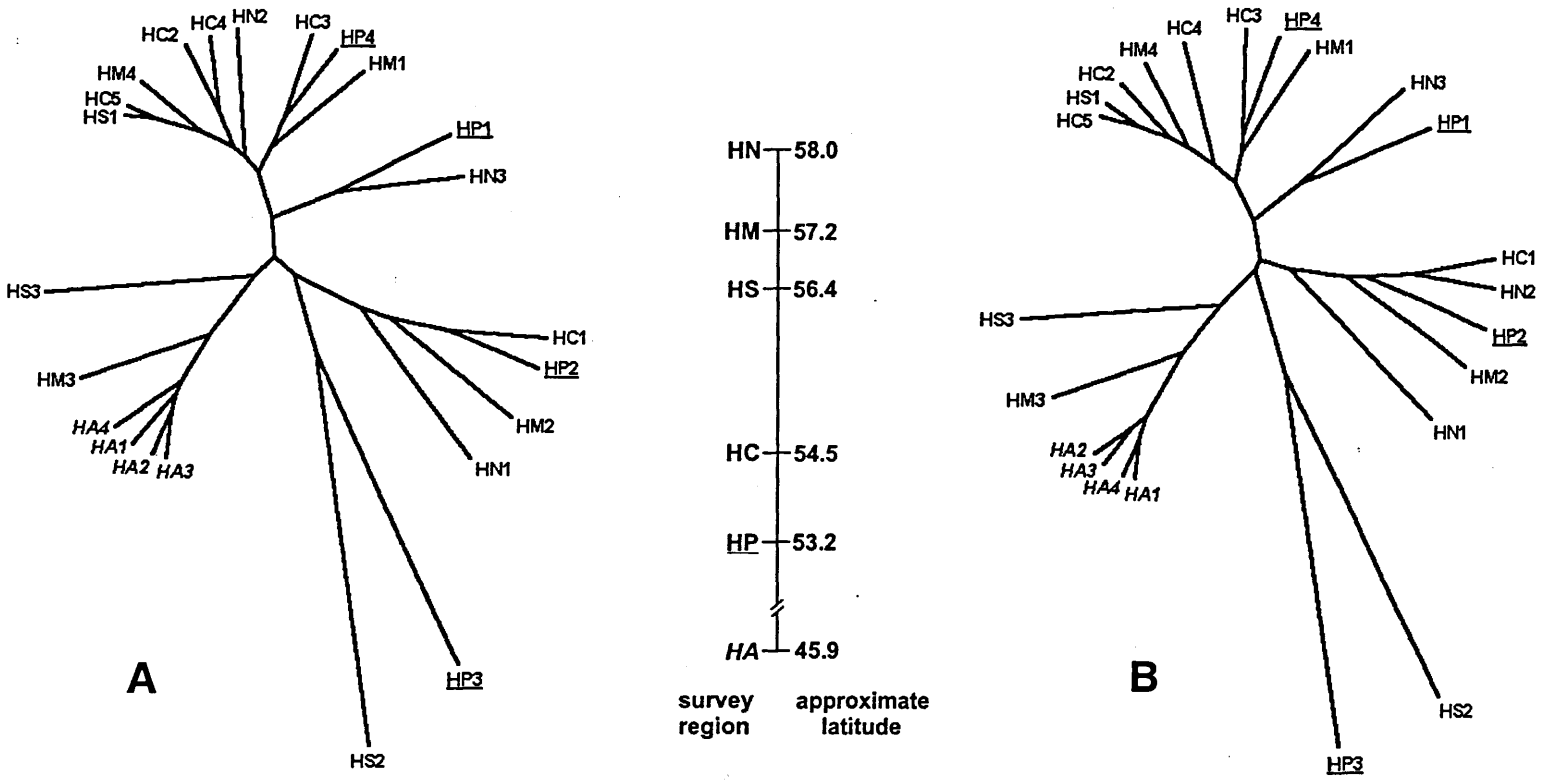

Fig. 5.2.3 Un-rooted tree from UPGMA cluster analysis based on Nei's (1972) genetic distance between *C. heterophyllum* populations. Branch lengths are scaled relative to the maximum genetic distance between populations. Analyses are of ramets (A) and genets (B). Peripheral populations are underlined, Swiss and Italian populations are shown in italics. Key shows approximate latitude of survey regions, accurate site locations are given in Table 1.3.1.

A significant correlation between genetic distance and geographic distance is seen in *Cirsium acaule* ($R^2 = 0.56$, $P < 0.005$, Fig. 5.3.1) and a weak but significant correlation in *C. arvense* (Ramets: $R^2 = 0.14$, $P < 0.0001$, Fig. 5.3.2a; Genets: $R^2 = 0.09$, $P < 0.005$, Fig. 5.3.2b). No correlation between genetic distance and geographic distance is seen in *C. heterophyllum* either when the analysis includes all populations surveyed or only those occurring in the UK.

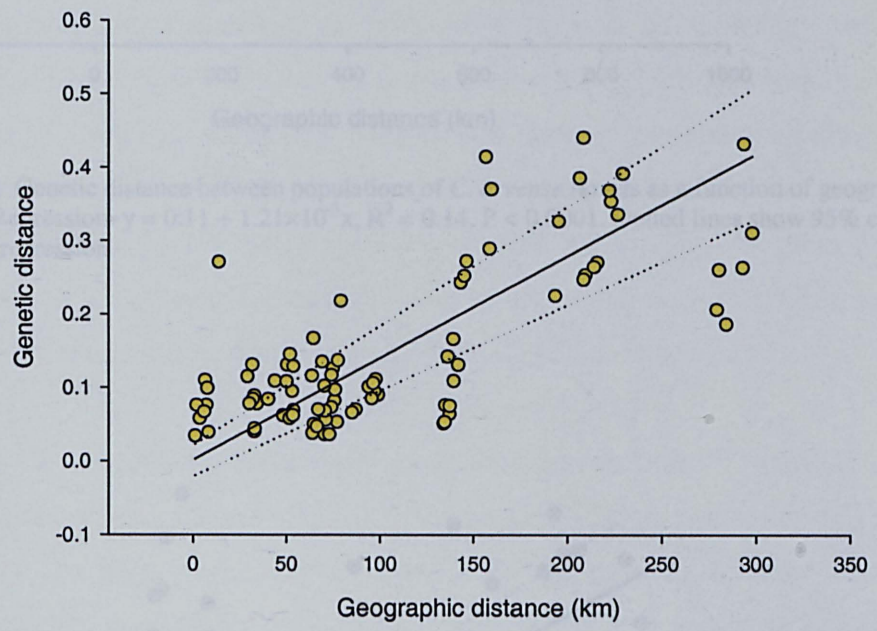

Fig. 5.3.1 Genetic distance between populations of *C. acaule* as a function of geographic distance. Regression: $y = 1.35\times10^{-3} + 1.39\times10^{-3}x$, $R^2 = 0.56$, $P < 0.005$. Dotted lines show 95% confidence interval of regression.

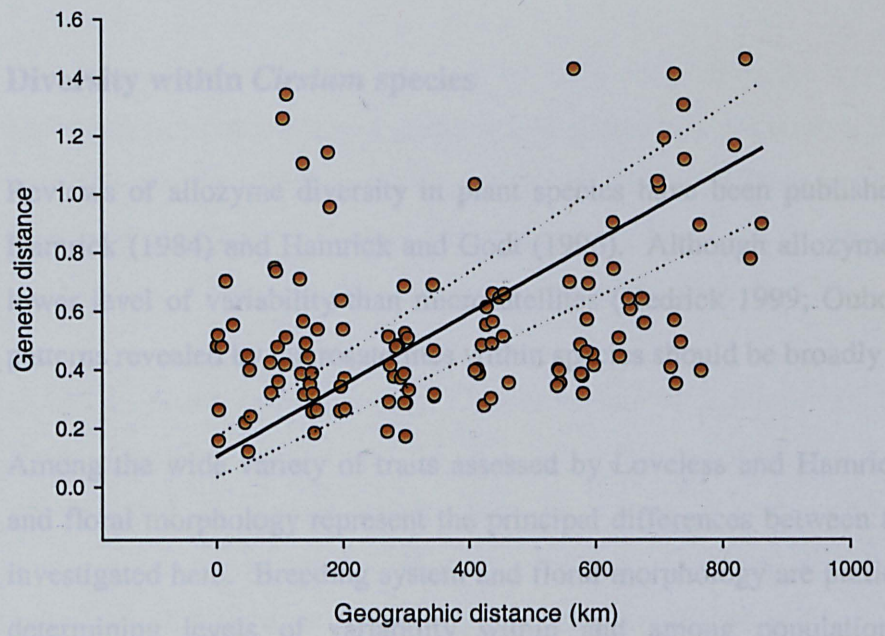

Fig. 5.3.2a Genetic distance between populations of *C. arvense* ramets as a function of geographic distance. Regression: $y = 0.11 + 1.21\times10^{-3}x$, $R^2 = 0.14$, $P < 0.0001$. Dotted lines show 95% confidence interval of regression.

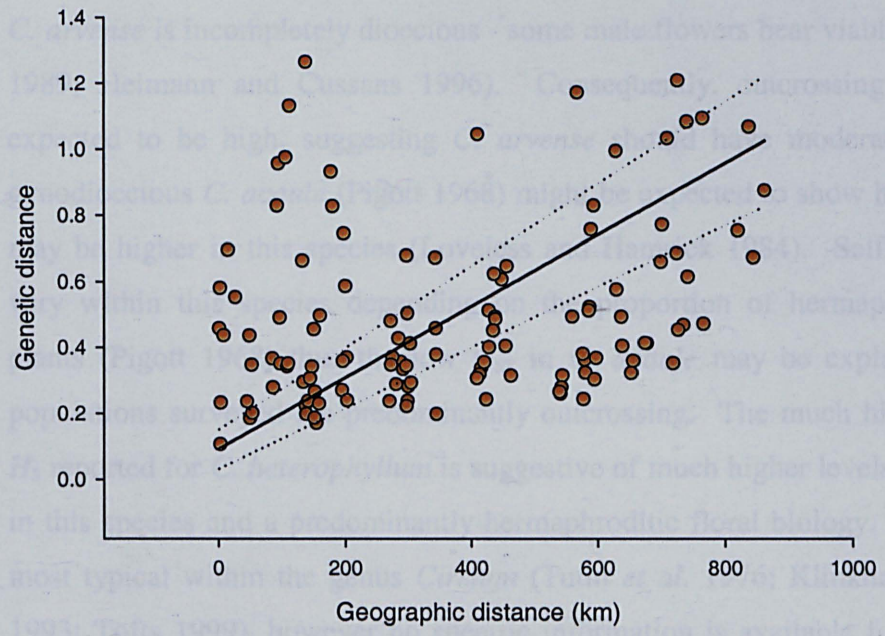

Fig. 5.3.2b Genetic distance between populations of *C. arvense* genets as a function of geographic distance. Regression: $y = 0.92 + 1.08\times10^{-3}x$, $R^2 = 0.09$, $P < 0.005$. Dotted lines show 95% confidence interval of regression.

5.4.1 Discussion

Diversity within *Cirsium* species

Reviews of allozyme diversity in plant species have been published by Loveless and Hamrick (1984) and Hamrick and Godt (1996). Although allozymes generally show a lower level of variability than microsatellites (Hedrick 1999; Ouborg *et al.* 1999) the patterns revealed by microsatellites within species should be broadly comparable.

Among the wide variety of traits assessed by Loveless and Hamrick, breeding system and floral morphology represent the principal differences between the *Cirsium* species investigated here. Breeding system and floral morphology are particularly important in determining levels of variability within and among populations, although many characteristics of a species history and ecology are also likely to have an effect (Loveless and Hamrick 1984; Hamrick and Godt 1996). G_{ST} is generally lower in outcrossed species when compared with those with higher rates of selfing, likewise G_{ST} is lower in hermaphroditic species compared to dioecious species.

C. arvense is incompletely dioecious - some male flowers bear viable seed (Grime *et al.* 1989; Heimann and Cussans 1996). Consequently, outcrossing in this species is expected to be high, suggesting *C. arvense* should have moderately low G_{ST}. The gynodioecious *C. acaule* (Pigott 1968) might be expected to show higher G_{ST} as selfing may be higher in this species (Loveless and Hamrick 1984). Selfing rate is likely to vary within this species depending on the proportion of hermaphrodite and female plants (Pigott 1968) thus the low G_{ST} in *C. acaule* may be explained in part if the populations surveyed are predominantly outcrossing. The much higher G_{ST} and lower H_S reported for *C. heterophyllum* is suggestive of much higher levels of self-fertilisation in this species and a predominantly hermaphroditic floral biology. Hermaproditism is most typical within the genus *Cirsium* (Tutin *et al.* 1976; Klinkhammer and de Jong 1993; Tofts 1999), however no specific information is available for *C. heterophyllum* and it is therefore uncertain whether hermaphrodites of this species would be self-compatible.

Deviation from Hardy-Weinberg equilibrium

The Hardy-Weinberg principal can be used to predict the expected frequency of genotypes from the observed frequency of alleles in a population. If the observed and expected genotype frequencies do not differ significantly they are said to be in Hardy-Weinberg equilibrium (HWE). Deviations from HWE will occur when the underlying assumptions of the Hardy Weinberg principle are violated (see Hartl 1988).

Unlike *C. acaule*, populations of *C. arvense* and *C. heterophyllum* do not conform to Hardy-Weinberg equilibrium (Table 5.3.2). The majority of populations in both *C. arvense* and *C. heterophyllum* show an excess of heterozygotes, an excess of homozygotes is seen in relatively few populations. Large deviations from HWE are typical of species with high levels of clonal reproduction (Ivey and Richards 2001; Uthicke *et al.* 1998, 1999, 2001; Vasseur 2001) and consequently F_{IS} may not be a reliable indicator of breeding system (inbreeding v outbreeding). Bias towards heterozygote excess at both the ramet and genet level of analysis may be explained by heterozygote advantage (Lesica and Allendorf 1992, 1995; Oostermeijer *et al.* 1994) combined with clonal selection (a gradual loss of genotypes due to attrition, so only those genotypes that produce vigorous clonal growth remain; Schaal and Leverich 1996).

Geographical structure of genetic diversity

There is little agreement between the three *Cirsium* species when patterns in population structure and diversity are considered. At the largest scale, increasing geographic distance between populations should be expected to result in decreasing genetic similarity (isolation by distance). This is likely to result from both historical patterns resulting from post-glacial migration (Gabrielsen *et al.* 1997; Tremblay and Schoen 1999) and the effects of decreasing contemporary gene flow between increasingly distant populations (Schaal and Leverich 1996). Isolation by distance is seen in both *Cirsium acaule* (Fig. 5.3.1) and *C. arvense* (Fig. 5.3.2a,b), yet *C. heterophyllum* shows no such relationship. In accordance with predictions based on the increased isolation of populations at the range edge (Schaal and Leverich 1996; Ellstrand and Elam 1993),

peripheral populations of *C. acaule* are divergent both from each other and from those in core areas of the species range.

In *C. heterophyllum* there is no geographic structure when genetic distances between populations are visualised as a tree diagram (Fig. 5.2.3A,B). Populations of *C. heterophyllum* from the Swiss and Italian Alps show greatest genetic similarity to several Scottish populations, despite the fact that these are the most remote geographically. Tree diagrams for *C. acaule* (Fig. 5.2.1) and *C. arvense* (Fig. 5.2.2A,B) demonstrate much greater geographic structure, with populations from nearby survey areas typically being grouped close to each other in the tree. Outlying populations indicated by the *Cirsium acaule* tree diagram are those that occur at the edge of the species range, where this species occurs with lowest frequency (Fig. 2.3.5, 2.3.6). In *C. arvense* also, outlying populations are those that were sampled in the areas of its range where its frequency is lowest (central and north-west Scotland, Fig. 2.3.5), thus population isolation is implicated in promoting population divergence in both species.

Much of the British flora is likely to have been eliminated during the Quaternary glaciations, with subsequent re-colonisation from southern refugia during milder periods (Pigott 1954; Godwin 1975). Species would have gone through a series of range expansions and contractions, the last of which would have generated the native biological diversity evident today (Taberlet *et al.* 1998; Scotti *et al.* 2000). Some species present in the contemporary UK flora may have persisted in periglacial areas of southern England during the most recent (Weichselian) glaciation (Godwin 1975; Conolly and Dahl 1970). The colonisation of the British mainland by species not persisting during the Weichselian glaciation is most likely to have occurred from refugia in continental Europe via northern France (Taberlet *et al.* 1998; Hewitt 2000).

Range expansion from refugia would have involved a northward spread of the leading edge of the species, a process that implies successive bottlenecks, thereby reducing genetic diversity in more northerly populations (Taberlet *et al.* 1998). The genetic similarity between colonising and refugial populations will decline as colonisation distance increases due to bottlenecks and genetic drift. Gene flow between established populations is likely to reduce such differences, however, since the magnitude of gene flow between populations is a function of distance, isolation by distance is likely to

persist. Isolation by distance is a common finding in studies of geographic patterns in plant population structure (eg: Dumolin-Lapègue *et al.* 1997; Gabrielsen *et al.* 1997; Tollefsrud *et al.* 1988; Tremblay and Schoen 1999).

When compared with *Cirsium acaule* and *C. arvense*, the lack of isolation by distance and the absence of geographic structure to genetic variation in *C. heterophyllum* is intriguing. Gabrielsen *et al.* (1997) found little geographic structure in UPGMA analysis of *Saxifraga oppositifolia* populations in Norway and Svalbard. Populations from different geographical origins were to a large extent intermingled, although isolation by distance was evident in this species. Gabrielsen *et al.* (1997) postulate that recent long distance dispersal is responsible for the absence of clear geographic structure in this species, Tollefsrud *et al.* (1988) also find evidence for recent long distance dispersal in *Saxifraga cespitosa.*

Recent long distance dispersal is unlikely to explain the lack of geographic structure in *Cirsium heterophyllum.* Long distance dispersal events would need to predominate in order to essentially randomise the geographic structure in this species (Fig. 5.2.3), furthermore, such events would need to cover distances as great as 1500km (the distance between the Alpine and similar central Scottish populations). This would prevent such pronounced population differentiation (G_{ST} = 0.318 – 0.359). If such events were rare, much greater local structuring would be expected in many areas – such as the close clustering of the four Alpine populations of *C. heterophyllum.*

An alternative explanation for the disruption of isolation by distance could be an historic long distance dispersal event from Scandinavia. Colonisation of the UK might then have occurred from both ice-free refugia in the north (via Scandinavia; see Tollefsrud *et al.* 1998 and references therein) and the south (via southern England). UK populations separated by relatively small geographic distance could then have been established from very different geographic origins and show a large genetic distance between them. An additional consequence of this theory would be the existence of a 'suture zone' between the two groups of populations – where hybridised populations are typically characterised by higher genetic diversity relative to either source group. Hewitt (2000) lists such a zone in Scotland, though the data for *C. heterophyllum* do not

suggest a Scottish suture zone is present in this species. Additionally, local populations would still be expected to cluster in the UPGMA analysis, which they do not.

Perhaps the most likely explanation for the lack of geographic structure in *C. heterophyllum* is that of an extremely rapid expansion of the species, followed by essentially random divergence of established populations by genetic drift. Pollen record estimates suggest some forest trees may have spread from glacial refugia at up to 2000 m/y (Bennet 1986). Scotti *et al.* (2000) suggest *Picea abies* (Norway spruce) may have reached its maximum range extension in only 250 – 500 generations. They find no evidence for isolation by distance and suggest that this period may not have been long enough to allow population divergence in *P. abies.* It is quite possible that the wind dispersed *C. heterophyllum* could rival this rate of spread. Comes and Abbott (1998) cite historical long distance dispersal and rapid range expansion as the likely cause for a lack of isolation by distance or geographic structure of allozyme variation in *Senecio gallicus. S. gallicus*, like *C. heterophyllum* and many other members of the Asteraceae, is well adapted for dispersal by wind. The spatial structure of allozyme variation in *S. gallicus* in the Iberian Peninsula and southern France is almost randomised, a similar pattern to that seen in *Cirsium heterophyllum* in the UK. Although little spatial structure was reported for allozyme variation in *S. gallicus* this was not the case for cpDNA or RAPD variation (Comes and Abbott 1998,2000), suggesting it would be advisable to determine whether spatial structure in genetic variation in *C. heterophyllum* might be detected by alternative molecular markers.

Despite the lack of geographic structure in allozyme variation in *S. gallicus*, population differentiation is moderately high (F_{ST} = 0.151; Comes and Abbott 1998). A similar pattern is seen in the submediterranen mountain plant *Anthyllis montana* L., which is believed to have experienced 'distributional stasis' and range fragmentation (Kropf *et al.* 2002). Low levels of contemporary gene flow between local populations are believed to responsible for promoting population divergence and maintaining the lack of spatial structure in both *S. gallicus* and *A. montana* (Comes and Abbott 1998; Kropf *et al.* 2002). The lack of geographic structure of genetic variation observed in *C. heterophyllum* could well result from similarly rapid migration, followed by habitat fragmentation. The consequent isolation of populations would then promote their divergence, largely through mutation and genetic drift (Kropf *et al.* 2002).

Genetic diversity in peripheral populations

Populations at the edge of a species range may show low genetic diversity relative to core populations as a result of both historic and contemporary processes. Populations are expected to show a decrease in genetic diversity with increasing distance from glacial refugia, reflecting an increase in population bottlenecks during range expansion (Ibrahim *et al.* 1996; Kropf *et al.* 2002). Consequently, a decline in genetic diversity across a geographic range is likely to indicate the species migration route (Stone and Sunnucks 1993; Petit *et al.* 1997; Kropf *et al.* 2002). Although such a decline is most likely to be toward the leading [northern] edge of a species expansion (Kropf *et al.* 2002), lower genetic diversity may also be seen at the retreating [southern] edge. At the retreating edge, diversity may be lower as shrinkage, dissection and extinction of populations is likely to result in the remaining populations being severely bottlenecked (Hewitt 2000; Walter and Epperson 2001). Current isolation of many peripheral populations is predicted to result in low genetic diversity due to population bottlenecks and genetic drift (Schaal and Leverich 1996; Lesica and Allendorf 1995). Reduced genetic diversity in populations at the edge of a species range is reported often (eg: Eckert and Barrett 1993; Dumolin-Lapègue *et al.* 1997; Lammi *et al.* 1999; Petit *et al.* 1997).

Of the three *Cirsium* species investigated, only *C. acaule* shows a decline in diversity from core to peripheral areas of its geographic range. It may not be possible to determine whether this pattern results primarily from the contemporary isolation of these peripheral populations (Chapter 2) or is a relic of the species' migration. The likely explanation is a combination of the two. The decline in diversity across the UK range of *C. acaule* suggests that this is not merely a result of the increased isolation of peripheral populations.

Levels of genetic diversity at the southern range edge of *Cirsium heterophyllum* would result from conflicting processes. Since colonisation of the UK is likely to have occurred via southern England (Conolly and Dahl 1970; Taberlet *et al.* 1998; Hewitt 2000), its southern range limit in the UK is likely to represent the retreating edge of this species' migration. If diversity declines with distance from a refugium, then more

northerly populations are expected to be less diverse (Petit *et al.* 1997; Kropf *et al.* 2002). Conversely, the southern periphery is expected to show lower diversity due to the effects of increased population isolation (Lesica and Allendorf 1995; Schaal and Leverich 1996; Hewitt 2000). As a result, any decline in diversity might be difficult to detect without sampling a very large number of populations.

If *C. heterophyllum* colonised the UK as rapidly as the geographic structure of its genetic variation suggests, it is not likely that a migration-induced decline in genetic variation toward either latitudinal limit will be evident. Geographically peripheral populations of *C. heterophyllum* would be expected to be less diverse, due predominantly to the effects of decreased population size and increased isolation. Although there is no apparent decline in population size approaching the southern periphery of *C. heterophyllum* (Table 5.3.1c), populations do become more isolated (Chapter 2). The effect of contemporary isolation on peripheral populations of *C. heterophyllum* might not be sufficient to result in decreased diversity in these populations. The loss of diversity in isolated populations is likely to be slowed in clonally reproducing plants due to retention of diversity through clonal persistence and increased opportunity for sexual reproduction of long lived clones (Schaal and Leverich 1996; Young *et al.* 1996; Ayers and Ryan 1997). Furthermore, gene flow may not be of major importance in maintenance of diversity in clonally reproducing species, although the maintenance of diversity in such species is not well understood (Ellstrand and Roose 1987).

Although *Cirsium arvense* does not reach a geographic limit within the UK, a decline in genetic diversity with increasing latitude might still be expected due to persistent effects of post-glacial migration. No such pattern is seen in this species. Throughout history *C. arvense* shows a close association with the activity of man (Godwin 1975; Moore 1975). This species is typical of disturbed areas such as waysides and is a serious weed of pastures and arable fields (Moore 1975; Grime *et al.* 1989; Rose 1991); it is characterised by frequent extinction and colonisation events. *Cirsium arvense* is extremely frequent throughout Britain when surveyed at differing spatial scales (Chapter 2). Factors determining levels of genetic variation within *C. arvense* populations may be particularly complex. Isolation by distance is very weak in this species although geographic structure is evident, it may be that long distance dispersal

(whether natural or anthropogenic) has weakened any historic patterns. Rapid colonisation by *C. arvense* of many new areas has been attributed largely to coincidental spread by man along with crop seeds, cattle etc. (Moore 1975; Heimann and Cussans 1996). It may not be possible to draw firm conclusions about geographic patterns in genetic diversity in *C. arvense* without more extensive sampling.

Clonal diversity

There is no relationship between clonal diversity and latitude in either *Cirsium heterophyllum* or *C. arvense*. Mean levels of clonal diversity (D, Table 5.3.1) in *C. heterophyllum* and *C. arvense* are typical of those found in species which regularly produce sexual progeny in addition to vegetative reproduction (Ellstrand and Roose 1987). *Cirsium arvense* shows a much greater range diversity (D = 0.97 – 0.08) compared with *C. heterophyllum* (D = 1 – 0.42). *Cirsium arvense* exhibits one of the widest ranges of clonal diversity reported for any plant species (Ellstrand and Roose 1987, Eckert and Barett 1993, McClintock and Waterway 1993). In contrast with *C. heterophyllum*, some populations of *C. arvense* appear to have been established almost exclusively by vegetative reproduction. It is possible however that the number of genotypes in *C. arvense* has been underestimated due to the small number of loci used for genotyping individuals of this species (Eckert and Barett 1993).

Eckert and Barett (1993) report decreased clonal diversity at the northern periphery of the aquatic herb, *Decodon verticillatus*. Low clonal diversity in peripheral populations of *D. verticillatus* is believed to result mainly from ecological factors that reduce seed production and establishment to negligible levels. Northern peripheral populations are maintained almost exclusively by clonal reproduction (Eckert and Barett 1993). Even occasional sexual reproduction could maintain high diversity within predominantly clonal populations (Schaal and Leverich 1996). The high diversity in some peripheral populations of *C. heterophyllum* is likely to result from past incidences of sexual reproduction together with long life of the resulting offspring.

The geographical distribution of genetic diversity seen in *Cirsium acaule* supports the expectation that peripheral populations often have low genetic diversity and are

genetically divergent. The absence of such a pattern in *Cirsium heterophyllum* suggests that this is not a general rule. Contemporary patterns of intraspecific genetic diversity result from a complex interaction of historical, ecological and anthropogenic factors. It may be misleading to make assumptions about the geographical pattern of genetic diversity within a species based solely on the present day distribution of its populations.

Chapter 6: General discussion

6.1.1

Plant reproduction is the only character to vary with latitude approaching the range edge of *Cirsium acaule*, *C. eriophorum* and *C. heterophyllum*. All of these species show a decline in reproductive potential toward the range edge, whether in terms of seed production per capitulum or flowers per plant (Chapter 4). No relationship with latitude is seen in the vegetative growth of ramets of these species. Populations of *Cirsium acaule* and *C. heterophyllum* become more isolated as the range edge is approached; the data for *C. eriophorum* are not adequate to allow investigation of this pattern (Chapter 2). None of these species occurs in atypical habitat at the periphery when compared with populations from the core areas of their UK distribution (Chapter 3). The widespread *C. arvense* does not show a relationship between latitude and any of the characters measured.

Climate and regeneration

Many studies report a reduction in seed production at the edge of a species geographic range (Pigott 1968; Pigott and Huntley 1981; Reinartz 1984b; Eckert and Barrett 1993; García *et al.* 2000; Dorken and Eckert 2001). The reproductive phase of the plant lifecycle shows particular sensitivity to climate (Marshall 1968; Pigott 1968; Pigott and Huntley 1981; Houle and Filion 1993; Despland and Houle 1997; Woodward 1997; García *et al.* 2000). Sensitivity to climate is indicated by the decline in reproductive potential displayed by *Cirsium acaule*, *C. eriophorum* and *C. heterophyllum* and the close association between their range boundary and isotherms of summer temperature (Pigott 1968; Conolly and Dahl 1970; Tofts 1999). This supports the assertion that climate is of primary importance in determining species distributions (Woodward 1996).

Despite the frequent decline in seed production, recruitment by seed is observed in peripheral populations since pronounced interannual variation in both climate and seed production occurs (Pigott 1968; Pigott and Huntley 1981; Houle and Filion 1993;

Despland and Houle 1997; Woodward 1997). Production of viable seed does not necessarily result in the expansion of a species current geographic range, since recruitment depends on seed production, seedling establishment and seedling survival (Pigott 1992; Dorken and Eckert 2001). The quantity of seed produced in peripheral populations may not be sufficient for regeneration to occur (Pigott 1992). Dispersal may limit plant distribution even given high seed production and availability of favourable microsites (Primack and Miao 1992).

If the occurrence of favourable microsites decreases approaching the periphery (as discussed in Chapter 2), then expansion of a species' range may become increasingly unlikely given the combined effects of a decrease in seed production (Chapter 4) and the decline in seed dispersal with increasing distance from the parent plant (Pigott 1992; van Dorp *et al.* 1996). Pigott (1992) suggests that a species' present day limit occurs at the point along a climatic gradient where frequency and quantity of seeds drops below that required for regeneration. Failure to produce fertile fruit (eg. *C. heterophyllum*) is a characteristic of relict species which are no longer in equilibrium with present climate (Woodward 1990; Pigott 1992). This emphasizes the importance of the history of a species in relation to understanding the control of its present geographic limits.

Eckert and Barrett (1993) report an increase in the relative importance of clonal reproduction at the northern periphery of *Decodon verticillatus*. This is due to a combination of climatic and genetic factors that decrease seed production and seedling survival in northern populations, whereas clonal propagation occurs throughout the species range. An absolute increase in clonal reproduction in *C. acaule* is suggested by increasing clump size toward its northern limit (Chapter 4). A similar response has been reported at both high latitude (Mooney and Billings 1961) and high altitude (Whitlock 1983), although no such pattern is seen at the southern limit of *C. heterophyllum* when clone size is estimated using molecular markers (Chapter 5).

Vegetative offspring may have a higher chance of establishment in peripheral populations when compared with plants produced by seed (Eckert and Barrett 1993). This raises interesting questions about whether the pattern seen in *C. acaule* arises as a result of reproductive assurance (see Pannell and Barrett 1998) or a decrease in the lifespan and hence the size (Pigott 1968; Whitlock 1983) of individuals in the more

southerly populations. As the range edge of both *C. acaule* and *C. heterophyllum* is approached, a decreasing proportion of flowering individuals within a population produce viable seed (Chapter 4). Given the effects of resource limitation proposed by Stephenson (1981), determining the relationship between genet size and seed production of individual plants would allow investigation of potential trade-offs between clonal and sexual reproduction.

Reproduction, isolation and genetic variation

Range-wide patterns of genetic variation do not show much agreement between species (Chapter 5). Only *C. acaule* shows a decline in genetic variation approaching the range edge, as predicted by Pigott (1968) based on morphological characteristics. *C. heterophyllum* shows similar levels of genetic variation throughout its UK latitudinal range and has little geographic structure. *Cirsium eriophorum* was not included in analyses of population genetic structure.

Decreased genetic variation and increased population divergence is expected at the range edge of both *C. acaule* and *C. heterophyllum* given the decline in seed production (Chapter 4). The increasing proportion of individuals failing to set seed at the periphery of both species suggests the recruitment should occur from fewer genetic individuals. Increased isolation of peripheral populations of these species (Chapter 2) is also expected to result in decreased diversity and increased divergence of peripheral populations. This pattern of population genetic structure is observed in *C. acaule*, as predicted by Pigott (1968) based on morphological characters. In *C. heterophyllum* no such pattern is evident. It may be that clonal reproduction (which allows plants to survive indefinitely; Moore 1975; Pigott 1992) and occasional reproduction by seed (Ellstrand and Roose 1987) interact to maintain levels of genetic variation within peripheral populations of this species, despite the predictions of Hewitt (2000) and Walter and Epperson (2001) that diversity may decline.

Geographic structure of genetic variation and species history

Comparability between species is maximised by comparing species with maximum evolutionary relatedness (Kelly and Woodward 1996; Silvertown and Dodd 1996; Blackburn and Gaston 1998). This makes it possible to avoid comparing apparently similar species that nonetheless have a very different evolutionary history. The differences in the geographic structure of genetic variation in *C. acaule, C. arvense* and *C. heterophyllum* are unlikely to be contemporary effects resulting from major differences in biology. These species share most key traits considered important in determining patterns of genetic structure within species (Loveless and Hamrick 1984; Hamrick and Godt 1996). Given the great similarity between these species and their patterns of reproductive potential and population isolation, differences in geographic structure of intraspecific genetic variation between these species are surprising.

Cirsium acaule, *C. arvense* and *C. heterophyllum* differ both in their distribution throughout the UK (Chapter 1) and the communities in which they occur (Chapter 2). According to Clapham *et al.* (1981) and Rose (1991), the southern species, *C. acaule*, occurs in short calcareous grasslands. The widespread *C. arvense* occurs in grasslands, wasteground, waysides, and arable fields, whilst the northern *C. heterophyllum* is listed as occurring in upland meadows, grasslands, waysides and open woodland. With the exception of *Cirsium heterophyllum* and *C. palustre*, *Cirsium* species that occur in the UK are absent from woodland (Clapham *et al.* 1981; Rose 1991), where they may show severely reduced reproduction and competitive ability (Moore 1975; Grime *et al.* 1989; Klinkhamer and de Jong 1993; Tofts 1999). A partial explanation for marked differences in the geographic structure of genetic variation within these species may lie in the relationship between their habitat preferences and likely post-glacial history.

Large areas of southern and eastern England are believed to have remained free of ice during the most recent (Weichselian) glaciation. Tundra vegetation characterised these periglacial areas, although the diversity of habitat and microclimate in the periglacial area may have allowed persistence of a variety of forb species not typically associated with tundra. *Salix herbacea* and birch (*Betula*) scrub occurred in the UK during the late Weichselian (pollen zones I – III, 15000 – 10000 years before present, yBP) although

open vegetation predominated with little forest cover. The predominantly sub-arctic climate during this time would have excluded thermophilous species. Closed forest developed over most of the UK during the much warmer conditions of the early post-glacial (Flandrian) period (zone IV, 10000 yBP) and persisted until cleared by man (zones VIIb and VIII, 5000 – 3000yBP). Many light demanding species present previously in the British flora are absent from pollen records during the forest maximum and do not reappear until after the late Flandrian forest clearance (summarised from West 1970 and Godwin 1975).

Godwin (1975) presents palaeobotanical evidence for a number of *Cirsium* species in the UK, identified both by fruit and pollen. There is evidence of *Cirsium arvense* in the closing stages of the Weichselian glaciation, although the evidence of this species in the Flandrian is not until later pollen stages (zones VIIb and VIII) approximately 5000 yBP. All late Flandrian records of *C. arvense* are associated with human settlement. *C. arvense* is typical of the species listed by Godwin as weeds and ruderals, the majority of which do not appear in the Flandrian record until 5000 yBP – after forest clearance has commenced.

C. heterophyllum is also present at the closing stages of the Weichselian glaciation. Other records for *C. heterophyllum* may include *C. palustre* also. These list *C. heterophyllum/C. palustre* as present in the UK continuously from the mid Weichselian (approximately 50000 - 20000 yBP). Godwin (1975) views *C. palustre* as 'a persistent native species, at least from the Weichselian time'. It is possible that this description may be appropriate for *C. heterophyllum* also, given the combined evidence presented for the presence of these species. *C. palustre* occurs in many of the habitats occupied by *C. heterophyllum*, including open woodland (Grime *et al.* 1988; Rose 1991). It is likely that both *C. heterophyllum* and *C. palustre* could have persisted during the forest maximum in the UK.

Godwin (1975) does not discuss any palaeobotanical records of *C. acaule*. Conditions suitable for the existence of this species might be indicated by the presence of species with similar habitat preferences and species with similar present day distributions. Species such as *Helianthemum nummularium*, *Lotus corniculatus*, *Scabiosa columbaria* and *Succisa pratensis* that occur commonly with *C. acaule* (Appendix A; Rodwell

1992b) are present in the UK flora throughout the Weichselian and Flandrian. The continuous presence of these species suggests that open habitat suitable for *C. acaule* may have persisted throughout the Flandrian, however, the climate of the late Weichselian and early Flandrian is not likely to have been suitable given the high temperature requirement of *C. acaule* (Pigott 1968). The majority of species with a current day UK distribution similar to that of *C. acaule* (eg. *Arctium lappa*, *Avena fatua*, *A. strigosa*, *Bryonia dioica*, *Carduus nutans*, *Picris echioides*, *P. hieracioides*; see Perring and Walters 1990) are not recorded until the late Flandrian (zones VII and VIII, 7000 – 3000 yBP; Godwin 1975). Like *C. acaule*, most of these species require open habitat and would have been unlikely to colonise the UK until after the extensive clearance of forest by man.

A comparison between *C. heterophyllum* and species with analogous northern UK distributions (eg. *Empetrum nigrum*, *Rubus chamaemorus*, *Trollius europaeus*, *Vaccinium uliginosum*, *V. vitis-idaea*) indicates continuous presence of these species in the UK flora from the late Weichselian and throughout the Flandrian, albeit with much contracted distributions. Some species associated with the present day *C. heterophyllum* communities (eg. *Fillipendula ulmaria*, *Rumex acetosa*, *Stachys sylvatica*, *Trollius europaeus*; Appendix A; Godwin 1975; Rodwell 1992ab) also show a continuous presence throughout the Flandrian. *F. ulmaria* was present throughout both glacial and interglacial cycles (Godwin 1975).

The lack of geographic structure in the genetic variation of *C. heterophyllum* is most likely to have occurred as a result of a rapid expansion followed by fragmentation of the range and divergence of isolated populations. The greater structure shown by *C. acaule* and *C. arvense* may be the result of a more gradual colonisation (Chapter 5). Suitability of climate and tolerance of shaded conditions during the post-glacial period may be key factors in shaping the differences between these species. The periglacial climate and community types are likely to have been analogous to those in many areas of the current range of *C. heterophyllum* (Godwin 1975), this species is widespread in present day tall herb/salix scrub communities in the Scandinavian sub-arctic (personal observation). The evidence provided by the distribution of species typical of the habitat and present day distribution of *C. heterophyllum* suggests that this species could have persisted

throughout the Weichselian glaciation and would then have been well placed to expand its range in the UK as glaciers retreated.

C. arvense and *C. heterophyllum* were both present in the UK flora at the end of the Weichselian. Low temperatures during this period suggest that *C. acaule* is most likely to have been absent. C *arvense* is likely to have been excluded from the majority of the UK as forest cover developed in the early Flandrian. *C. heterophyllum* would have been able to persist under these conditions although it would have been confined to more open woodland. The rise in temperatures during the Flandrian would have allowed entrance into the UK of more thermophilous species. Such species requiring open habitat (*C. acaule*) could then have colonised the UK following forest clearance in the late Flandrian. Forest clearance would also have permitted colonisation by weeds and ruderals such as *C. arvense* and re-expansion of shade tolerant species such as *C. heterophyllum.*

This tentative history for the spread of *Cirsium acaule*, *C. arvense* and *C. heterophyllum* would go some way to explaining the very different patterns of genetic structure that exist across their UK geographic range. Clearly, there is a need to date more precisely the expansion of these species that the genetic data reveal. Given the paucity of direct evidence in the palaeobotanical record, application of molecular clock techniques to internal transcribed spacer (ITS) sequence phylogenies (as described by Kropf *et al.* 2002) could provide one such method.

Peripheral populations and conservation

Peripheral populations are considered particularly valuable for conservation due to divergent genetic structure (Lesica and Allendorf 1995) and pre-adaptations to global climate change (Safriel *et al.* 1994). Safriel *et al.* (1994) suggests they should be treated as a 'biogenetic resource' used for the rehabilitation and restoration of damaged ecosystems. Peripheral populations may show similar levels of genetic variation and divergence even if they result from historic fragmentation of the species range or are not subject to significant gene flow from more central populations (see Chapter 5; Lesica and Allendorf 1995). Therefore assumptions about conservation value should not be based solely on the current geographic distribution of populations.

Major climatic changes in the past have been accompanied by plant species migration rather than mass evolution of tolerant genotypes (Bradshaw and McNeilly 1991; Huntley 1991). Many plant species are expected to show a major shift in distribution as a result of predicted global climate change (Huntley *et al.* 1995; Saetersdal *et al.* 1998; Bakkenes *et al.* 2002). Although long distance dispersal will be critically important in determining the rate at which species migrate (Leishman *et al.* 1992; Higgins and Richardson 1998; Ouborg *et al.* 1999), it is most likely that a species expansion will be driven by populations at the range edge (Taberlet *et al.* 1998). Consequently, geographically peripheral populations are likely to be at the forefront of a species migratory response to changes in global climate (Safriel *et al.* 1994). Individuals further toward the core must advance much more slowly as they must disperse into habitat patches that are already occupied by the expanded edge (Hewitt 2000). Habitat fragmentation due to the activities of man further restricts the natural migration of species (Bradshaw and McNeilly 1991; Hill *et al.* 1999; Collingham and Huntley 2000). The isolation of peripheral populations may have profound consequences if they must facilitate a species' migration, particularly if they are genetically depauperate.

6.1.2 Conclusion

Populations at the periphery of a species range are of particular interest as they may inform on the factors that limit species distributions. Additionally, peripheral populations are often considered of high conservation value due to the expectation that they will be genetically divergent from other conspecific populations. Despite a parallel decline in seed production and increased isolation of populations approaching the range edge, *Cirsium acaule* and *C. heterophyllum* show very different patterns of intraspecific genetic structure; divergence of peripheral populations is seen in *C. acaule* only. This disparity suggests that it is not advisable to make assumptions about the genetic structure of populations based on their current patterns of distribution and reproductive potential. The present day distribution of genetic variation within a species may be dependent largely on the pattern of its post-glacial migration. Consideration of a species' history is important in understanding its current patterns of distribution,

reproduction and genetic variation and will ultimately inform on a species' potential response to future global climate change.

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Appendix A

Plant community survey data for communities in which *Cirsium acaule, C. arvense, C. eriophorum and C. heterophyllum* occur

Table A.1 Species associated with *Cirsium acaule*

	Site code																													
	AD1A	AD1B	AD2A	AD2B	AD3A	AD3B	AW1A	AW1B	AW2A	AW2B	AW3A	AW3B	AB1A	AB1B	AB2A	AB2B	AB3A	AB3B	AG1A	AG1B	AG2A	AG2B	AG3A	AG3B	AP1A	AP1B	AP2A	AP2B
Achillea millifolium	.	.	20	44	.	.	24	24	.	.	.	.	4	4	.	12	.	.	36	.	.	.	.	.	.	.	.	.
Bellis perennis	.	.	.	.	.	.	.	.	.	8	20	8	24	20	56	.	.	.	.	.	.	.	.	12	.	.	.	.
Blackstonia perfoliata	.	.	.	.	4	.	.	.	.	.	.	4	.	.	.	.	.	.	16	8	.	4	4	.	.	.	.	.
Campanula glomerata	.	.	.	.	.	.	.	.	.	16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Campanula rotundifolia	.	.	12	32	4	12	.	.	.	.	.	.	.	.	.	.	.	.	20	.	.	.	24	8	12	.	12	12
Centaurea nigra	.	8	.	16	.	.	.	.	.	.	.	.	.	.	8	8	.	.	.	.	44	12	.	.	.	.	20	.
Centaurea scabiosa	.	.	.	.	.	.	.	.	.	.	.	.	.	4	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Cerastium fontanum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4	.	.	.	.	.	.	.	.	.	.	.
Cirsium acaule	12	32	16	4	4	.	.	.	.	12	.	.	12	24	16	16	4	.	48	8	44	40	20	16	.	.	48	.
Cirsium vulgare	4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Clinopodium vulgare	.	.	12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Conopodium majus	.	.	.	.	.	.	.	16	4	8	.	.	.	.	.	.	.	.	.	4	.	.	4	4	.	.	.	.
Euphrasia sp.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	12	.	.	.	.	.	.	40	.	.
Fillipendula vulgaris	.	.	.	.	.	.	.	.	16	16	.	4	.	.	.	.	.	.	.	.	.	.	24	12	.	.	.	.
Galium sterneri	.	.	48	32	.	.	4	.	.	.	.	4	.	.	.	16	40	16	.	.	.	.	.	.	4	.	.	24
Galium verum	.	20	.	.	4	.	72	52	.	.	20	36	.	.	.	28	24	24	12	4	.	.	.	.	.	.	.	.
Geranium molle	.	.	.	.	.	.	.	.	.	16	8	.	.	.	8	.	.	.	.	.	.	.	.	.	.	.	.	.
Helianthemum nummularium	.	.	.	.	16	36	.	.	.	.	.	.	.	.	.	16	.	.	.	.	.	12	4	.	12	48	28	68
Heracleum sphondylium	.	.	.	.	.	.	.	.	.	.	16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hypochaeris radicata	32	20	16	24	.	12	4	24	.	16	.	.	.	.	.	.	48	28	.	.	56	28	36	24	.	.	.	.

Table A.1 (continued)

Leontodon autumnalis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	48	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Leontodon hispidus	48	16	68	44	4	16	36	40	36	28	36	12	100	28	24	.	16	68	.	.	.	.	.	.	12	20	20	8
Linum catharticum	.	.	12	.	.	.	.	.	.	.	.	4	.	.	.	.	8	44	.	4	4	8	.	8	28	4	.	.
Lotus corniculatus	24	36	36	56	.	28	.	.	24	28	28	20	72	100	20	16	100	52	92	56	76	36	40	28	36	40	.	24
Medicago lupulina	84	100	76	48	.	.	52	32	72	16	32	20	.	72	.	16	.	.	.	20	.	.	.	.	.	.	.	.
Ononis repens	16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	64	.	.	.	.	.	.	.	.
Origanum vulgare	.	.	.	.	.	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Pilosella officinarum	.	.	.	.	.	12	.	.	52	36	.	32	.	.	40	40	28	80	.	.	8	12	16	44	40	24	28	.
Plantago lanceolata	100	72	44	16	20	.	48	32	24	12	20	4	40	40	.	28	52	16	20	12	.	.	12	8	72	60	36	68
Plantago major	.	.	.	.	.	.	.	.	.	.	.	.	.	.	8	.	.	.	.	.	.	.	.	.	.	.	.	.
Plantago media	.	4	.	.	.	.	8	12	12	20	4	12	12	32	36	.	.	.	.	.	.	.	.	.	.	.	.	.
Polygala vulgaris	.	.	.	.	.	.	.	32	.	16	.	.	.	.	.	44	.	.	.	.	.	.	.	.	.	.	.	4
Potentilla erecta	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	12	.	.	16
Potentilla reptans	.	.	.	.	.	.	.	.	.	.	.	.	16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Primula veris	.	.	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Prunella vulgaris	.	.	24	.	.	.	4	.	.	.	16	16	28	12	52	.	76	44	.	.	.	.	.	.	.	.	36	40
Ranunculus acris	.	8	.	.	.	.	20	.	8	.	.	.	4	.	.	4	.	.	.	16	.	.	8	.	4	.	.	20
Rumex acetosa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4	.	.	.	.
Sanguisorba minor	.	.	24	4	28	44	4	.	56	84	.	.	.	68	88	72	44	44	.	8	100	100	36	44	80	80	60	72
Scabiosa columbaria	24	.	.	.	.	.	.	8	8	16	32	24	.	4	8	24	8	.	.	.	.	.	44	40	.	.	12	.
Securigera varia	.	.	.	.	64	4	.	.	.	.	12	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Senecio jacobea	.	4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4	.	4	.
Succisa pratensis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4	28	.	.	.	.	.	8	16	.	.	.	4

Table A.1 (continued)

Taraxacum sp. section Ruderalia	.	.	.	.	.	.	.	.	.	.	.	.	16	8	16	.	.	.	.	.	.	.	.	.	4	.	.	.		
Thymus polytrichus	.	.	.	.	12	20	.	.	32	60	.	.	.	.	.	.	12	40	44	32	4	40	16	60	84	96	80	68		
Trifolium pratense	.	16	.	.	.	.	92	96	.	.	.	4	8	.	32	64	.	.	.	.	.	.	.	.	.	.	.	8		
Trifolium repens	52	8	32	16	.	16	24	20	.	.	60	28	24	12	.	.	.	.	.	.	.	.	68	52	.	.	.	12		
Viola riviniana	.	.	.	.	16	.	.	.	.	4	.	.	.	.	.	.	.	.	.	.	8	.	4	4	.	.	.	4		
Agrostis capillaris	.	.	48	68	.	16	48	32	.	8	.	28	92	64	20	24	36	20	80	52	.	.	.	.	24	24	.	.		
Agrostis stolonifera	.	.	20	36	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		
Anthoxanthemum odoratum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	12	.	.	.	.	.	.	.	.		
Arrhenatherum elatius	.	.	.	.	.	.	16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		
Brachypodium pinnatum	.	.	.	.	100	100	.	.	.	.	.	.	.	.	.	.	.	.	.	.	64	36	.	.	.	.	.	.		
Briza media	.	.	12	16	16	.	24	88	48	20	32	16	.	.	.	.	12	.	.	32	32	20	36	56	32	.	28	12		
Bromopsis erecta	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	8	.	.	.	.	.	.		
Bromus hordeaceus Ssp. hordeaceus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	12	.	.	.	.	.		
Carex caryophyllea	.	.	24	40	36	24	.	52	48	56	84	80	24	16	80	76	52	28	.	.	.	.	72	84	56	64	44	68		
Carex flacca	.	.	36	52	20	4	24	68	32	32	32	20	20	56	32	48	24	12	12	20	100	76	48	40	40	100	80	76		
Cynosurus cristatus	100	96	.	.	.	.	32	8	56	12	76	20	72	68	12	16	56	24	.	.	.	16	.	.	.	.	.	52		
Dactylis glomerata	8	.	16	.	.	4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		
Festuca sp.	100	100	100	100	100	100	100	100	88	76	100	100	96	100	92	100	100	100	100	100	72	84	100	100	100	100	100	92		
Helictotrichon pratense	8	.	28	20	.	.	.	40	36	16	.	.	.	16	16	.	4	44	64	16	16	.	.	.	60	36	28	.		
Holcus lanatus	.	.	.	.	.	.	16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		
Koeleria macrantha	.	.	8	.	.	.	12	20	8	28	.	.	20	4	.	.	.	.	.	.	.	.	.	.	4	12	16	4		
Lolium perenne	32	16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		
Luzula campestris	.	.	.	.	.	.	.	.	20	24	.	.	.	.	.	.	.	.	.	.	20	8	.	.	.	.	.	.		
Phleum bertolonii	.	.	.	.	.	.	32	80	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		
Poa pratensis	40	32	32	48	20	36	40	12	28	20	24	.	.	8	20	8	.	.	.	.	.	.	.	.	.	.	.	.		

Note. Abundance recorded as percent frequency of shoots in each 50cm × 50 cm quadrat. Site locations are given in Table 1.3.1.

Table A.2 Species associated with *Cirsium arvense*

	Site code																							
	RD1A	RD1B	RD2A	RD2B	RB1A	RB1B	RB2A	RB2B	RG1A	RG1B	RG2A	RG2B	RP1A	RP1B	RP2A	RP2B	RC1A	RC1B	RC2A	RC2B	RM1A	RM1B	RM2A	RM2B
Achillea millifolium	.	.	.	.	.	.	.	.	12	.	.	.	.	.	.	.	.	.	4	8	.	20	.	.
Aegopodium podagraria	.	.	.	.	.	.	.	.	.	.	.	80	.	.	.	.	.	.	.	.	.	.	.	.
Cirsium arvense	88	72	100	100	72	48	100	84	80	88	80	64	88	100	100	84	84	100	100	100	100	100	100	100
Cirsium vulgare	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	12	.	.	.	.	.
Conopodium majus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4	.	.
Crataegus monogyna	.	.	.	.	.	.	.	.	.	.	4	.	.	.	.	.	.	.	.	.	.	.	.	.
Cruciata laevipes	.	.	.	.	.	.	.	.	32	16	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Galium aparine	.	.	.	.	.	.	.	.	.	.	4	100	.	.	24	.	.	44	.	.	.	.	16	12
Galium sterneri	.	.	.	.	.	.	.	.	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Geranium robertianum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	24	.	.	.	.	.
Heracleum sphondylium	.	.	12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Lathyrus pratensis	.	.	.	.	.	.	.	8	.	.	.	.	.	.	.	.	20	.	.	.	16	.	.	.
Leontodon hispidus	.	.	.	.	.	16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Leucanthemum vulgare	.	.	.	.	.	.	.	.	.	4	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Plantago lanceolata	.	.	.	.	40	36	.	.	.	.	.	.	.	.	.	.	.	.	16	.	.	.	.	.
Plantago major	.	.	.	.	.	.	.	.	.	12	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Plantago media	.	.	.	.	16	.	.	.	.	.	20	.	.	.	.	.	.	.	.	.	.	.	.	.
Potentilla reptans	16	.	.	.	.	.	.	.	16	24	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Table A.2 (continued)

Ranunculus acris	.	8	.	.	.	20	.	.	.	.	.	.	.	.	.	.	.	.	.	.	32	.	.	.
Ranunculus repens	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4	16	100	84	.	.	.	.	.	.
Rubus fruticosus agg.	.	.	16	28	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Rubus idaeus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	72	80
Rumex acetosa	.	.	.	.	12	20	.	.	.	.	.	.	.	.	.	.	.	.	.	.	8	36	.	.
Rumex obtusifolius	8	4	24	60	.	.	.	.	.	.	.	.	.	.	8	.	.	.	.	.	.	.	.	8
Sonchus oleraceus	.	.	.	.	.	.	.	.	.	4	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Succisa pratense	.	.	.	.	16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Taraxacum sp. section *Ruderalia*	.	.	.	.	.	12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	16	.	.
Trifolium pratense	.	.	.	.	.	4	.	.	24	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Trifolium repens	.	.	.	.	.	4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Urtica dioica	.	.	16	20	.	24	.	16	.	.	.	.	44	.	.	20	16	.	.	.	.	.	.	.
Veronica chamaedrys	.	.	.	.	.	.	.	.	.	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Agrostis capillaris	100	100	.	.	60	64	72	72	.	28	.	.	.	.	56	24	.	.	.	.	60	80	.	.
Agrostis stolonifera	.	.	.	.	.	.	.	.	.	.	.	.	100	100	.	.	72	52	.	.	.	.	.	.
Arrhenatherum elatius	.	.	100	40	.	.	.	.	56	72	8	8	.	.	16	32	.	.	32	48	.	.	44	16
Bromus hordeaceus ssp. hordeaceus	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Cynosurus cristatus	.	.	.	.	32	16	.	8	.	.	.	.	24	8	72	48	.	.	.	.	.	.	.	.
Dactylis glomerata	.	.	32	.	.	16	.	.	.	.	12	.	.	.	44	.	52	72	.	.	.	.	.	.
Elytrigia repens ssp. repens	.	.	.	.	.	.	.	.	.	.	.	60	.	.	.	.	.	.	.	.	.	.	.	.
Festuca sp.	56	80	.	.	.	24	.	.	.	.	.	.	.	.	100	80	.	.	60	80	100	100	.	.
Holcus lanatus	80	32	.	.	100	100	100	52	72	84	.	.	32	12	80	56	36	28	.	.	32	16	.	8
Lolium perenne	.	36	16	.	24	16	32	.	.	.	.	.	100	100	.	.	.	.	.	.	.	.	24	.
Phleum bertolonii	16	.	.	.	32	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Note. Abundance recorded as percent frequency of shoots in each 50cm × 50 cm quadrat. Site locations are given in Table 1.3.1.

Table A.3 Species associated with *Cirsium eriophorum*

	Site code																													
	ED1A	ED1B	EW1A	EW1B	EW2A	EW2B	EW3A	EW3B	EB1A	EB1B	EB2A	EB2B	EB3A	EB3B	EG1A	EG1B	EG2A	EG2B	EG3A	EG3B	EP1A	EP1B	EP2A	EP2B	EP3A	EP3B	EY1A	EY1B
Achillea millifolium	.	.	.	.	.	.	.	.	.	.	.	.	.	8	.	.	.	.	.	.	.	.	.	.	.	.	4	40
Agrimonia eupatoria	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	16	.	.	.	.	.	.
Alchemilla mollis	.	.	.	.	.	.	.	.	.	24	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Anthriscus sylvestris	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	8	.	.	.	.	.	.	.
Bellis perennis	.	.	.	.	.	.	.	.	.	.	.	.	32	28	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Centaurea nigra	.	.	.	.	.	.	.	.	.	.	.	.	.	32	.	.	.	.	.	.	.	.	.	.	.	.	32	20
Cerastium fontanum	12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	24	.	.	.	.
Cirsium arvense	36	.	4	.	.	.	12	20	.	4	.	4	.	.	12	.	.	.	.	.	.	.	.	.	.	.	.	.
Cirsium eriophorum	.	16	24	4	.	.	.	.	.	16	.	.	36	.	12	.	.	16	12	.	20	8	4	.	60	32	12	.
Cirsium vulgare	.	.	.	.	.	.	.	.	.	.	8	.	16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Conopodium majus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4	.	.	.	.	.	.	.	.
Convolvulus arvensis	.	.	4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	24	12	.	.	.	.	.	.	.	.
Cruciata laevipes	.	.	.	.	.	.	.	.	.	.	.	.	.	.	8	12	80	100	.	.	52	4	.	.	.	.	.	.
Daucus carota	.	.	.	.	.	.	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Euphrasia sp.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	16	.	.	.	.	.
Fallopia convolvulus	.	.	.	.	.	.	100	16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fillipendula vulgaris	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	8	.	.	.	.	.	.	.	.	.
Galium aparine	.	.	.	.	.	.	.	.	.	8	.	.	.	.	.	.	68	.	.	.	.	.	.	.	.	.	.	.
Galium verum	.	.	.	24	.	.	24	.	.	.	.	.	.	.	.	.	.	.	24	.	.	.	.	.	.	.	.	.
Geranium columbinium	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	16	.	.	.	.
Geranium molle	.	.	.	.	.	.	.	.	.	.	.	16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Geranium robertianum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4	.	.	.	.	.	.	.	.	.	.	.

Table A.3 (continued)

Heracleum sphondylium	.	.	.	.	.	.	.	4	4	24	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	20	32
Hypericum perforatum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4	.	.	12	.
Hypochaeris glabra	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	16	4	.	.	.	.
Hypochaeris radicata	.	.	.	.	.	.	.	.	.	.	.	.	20	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Lathyrus pratensis	.	.	.	.	.	.	.	.	.	.	.	8	.	.	.	.	.	12	.	.	.	.	.	.	.	.	.	48	.
Leontodon autumnalis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	24	16	.	.	.	.	.	.	.	.
Lotus corniculatus	.	.	.	.	.	.	.	.	.	.	.	.	28	16	.	.	.	.	.	.	20	.	.	.	.	12	.	32	.
Medicago lupulina	.	.	.	.	.	.	.	.	.	.	.	.	28	.	.	.	.	.	.	.	.	4	.	60	96	.	.	.	.
Mercurialis perennis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	28	12	.	.	.	.	.	.
Origanum vulgare	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	24	24	.	.	8	.	.	.
Plantago lanceolata	8	.	.	.	.	.	.	.	.	.	.	12	.	.	.	12	.	.	12	36	.	.	.	.	.	.	12	.
Potentilla reptans	56	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	24	.	.	.	.	.	.	.	.	.	.	.
Potentilla sterilis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	16	4
Prunella vulgaris	.	.	.	.	.	.	.	.	.	.	.	.	68	4	.	.	.	.	.	.	.	.	.	32	8	.	.	.	24
Ranunculus acris	.	.	12	.	.	.	.	.	.	.	20	32	12	24	.	.	.	.	.	.	.	.	.	8	4	.	.	.	8
Ranunculus repens	.	.	.	.	.	16	.	.	.	.	.	.	.	.	.	8	.	.	.	.	.	.	.	.	.	.	.	.	.
Rhinanthus minor	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	16	8	.	.	.	.	.	.	.	.	.
Rubus fruticosus agg.	.	28	.	.	.	.	.	.	.	.	.	.	.	.	.	20	.	.	.	.	.	12	.	4	32	44	.	.
Rumex acetosa	.	.	.	.	.	.	.	.	4	.	.	.	.	.	.	.	.	.	16	4	.	.	.	.	.	.	.	.	.
Sanguisorba minor	.	.	.	.	.	.	.	.	.	.	.	.	.	24	.	.	.	.	.	24	.	.	.	.	.	.	.	.	.
Sanguisorba officinalis	.	.	.	.	.	.	.	.	4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Senecio jacobea	.	.	.	.	.	.	.	.	.	.	.	.	8	.	20	.	.	.	.	.	.	.	.	.	4	.	.	.	.

Table A.3 (continued)

Taraxacum sp. section *Ruderalia*	.	.	.	.	.	.	.	.	.	.	.	.	36	.	20	.	.	.	.	.	.	.	4	4	.	.	.	.	.	.
Teucrium scorodonia	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	20	.	.	.	20	52	8	16	28	12	.	.	.	.
Tragopogon pratensis	.	.	.	.	.	12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Trifolium pratense	.	.	52	.	16	8	.	.	.	.	.	.	.	20	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Trifolium repens	60	52	52	100	.	.	.	.	.	.	.	.	84	20	.	.	.	.	16	72	.	.	.	.	.	.	.	.	.	.
Urtica dioica	.	.	24	.	.	.	80	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4
Veronica chamaedrys	.	.	.	.	.	.	16	.	.	.	.	.	.	.	.	4	.	.	.	.	.	.	.	.	40	.	.	.	.	.
Vicia cracca	.	.	.	.	.	.	48	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Vicia sativa	.	.	12	20	.	.	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Viola riviniana	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	16	.	.	.	.	.
Agrostis capillaris	100	100	56	.	56	32	.	.	.	8	72	100	.	.	.	.	.	.	.	.	.	.	.	.	20	52	.	.	.	.
Agrostis stolonifera	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	56	80	.	.	28	8	.	.	44	24	.	.
Arrhenatherum elatius	.	.	24	100	100	100	100	100	100	52	.	60	.	.	68	80	100	40	40	.	32	84	28	12	100	76	100	100	.	.
Brachypodium pinnatum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	20	.	.	.	.	.	.	.	100	24	.	.	.	.
Briza media	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	24	.	.	.	.	.	.	.	.	.	.
Bromopsis erecta	.	.	.	.	.	.	.	.	16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Bromus hordeaceus Ssp. hordeaceus	12	.	12	16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Carex caryophyllea	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	16	.	.	.	.	.
Cynosurus cristatus	60	.	.	.	28	20	.	.	.	.	56	72	.	72	.	.	.	.	100	72	.	.	.	.	.	.	.	.	.	.
Dactylis glomerata	.	.	.	16	32	40	.	20	52	32	16	.	.	.	16	32	.	20	8	.	16	.	.	.	.	.	16	.	.	.
Elytrigia repens ssp. repens	.	.	.	.	.	.	.	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Festuca sp.	80	24	100	32	56	80	100	4	52	100	80	100	100	100	100	72	60	16	100	100	100	100	100	100	100	100	56	72	.	.
Holcus lanatus	52	28	.	36	100	100	.	.	100	40	60	24	.	.	.	.	.	40	.	.	48	32	32	16	24	36	24	12	.	.
Lolium perenne	32	100	28	24	.	.	.	.	.	.	.	16	60	32	.	.	.	.	24	60	.	.	.	.	.	.	.	16	.	.
Luzula campestris	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	16	16	.	.	60	.	.	.	.	.
Phleum bertolonii	.	24	.	.	.	40	.	.	.	.	24	32	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Poa pratensis	76	.	8	.	.	.	.	.	.	.	.	.	44	84	.	.	.	.	.	.	.	.	.	16	.	.	.	.	.	.

Note. Abundance recorded as percent frequency of shoots in each 50cm x 50 cm quadrat. Site locations are given in Table 1.3.1.

Table A.4 Species associated with *Cirsium heterophyllum*

	Site code																																				
	HP1A	HP1B	HP2A	HP2B	HP3A	HP3B	HP4A	HP4B	HC1A	HC1B	HC2A	HC2B	HC3A	HC3B	HC4A	HC4B	HS1A	HS1B	HS2A	HS2B	HS3A	HS3B	HM1A	HM1B	HM2A	HM2B	HM3A	HM3B	HM4A	HM4B	HN1A	HN1B	HN2A	HN2B	HN3A	HN3B
Achillea millifolium	.	.	.	.	.	.	.	.	.	36	.	.	.	.	32	.	.	20	.	.	.	.	.	.	.	.	12	.	.	.	.	.	.	.	.	.	.
Aegopodium podagraria	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	16	24	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Ajuga reptans	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	8	20	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Alchemilla mollis	.	.	.	.	.	.	.	.	12	.	.	.	.	.	.	.	.	.	.	.	.	12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Anthriscus sylvestris	.	.	4	16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	8	.	.	.	.	.	.	.
Calluna vulgaris	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	16	.	.	.	.	.
Cardamine pratensis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	12	.	.	.
Centaurea nigra	.	.	.	.	20	20	.	.	.	24	.	.	12	24	.	24	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Cirsium arvense	.	.	.	.	.	.	.	.	.	.	.	.	.	8	28	.	.	.	.	.	.	.	.	16	.	.	.	.	.	.	.	.	.	.	.	.	.
Cirsium heterophyllum	92	88	100	100	96	100	96	100	100	96	56	80	100	80	96	100	100	100	92	92	92	96	100	92	100	100	100	100	100	100	100	100	100	100	100	100
Conopodium majus	.	.	.	.	.	.	.	.	28	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Cruciata laevipes	.	.	.	.	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Epilobium montanum	.	.	.	.	.	.	.	.	.	.	.	.	.	8	.	.	12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fillipendula ulmaria	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	32	60	.	.	.	.	12	12	.	.
Fragaria vesca	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	20	28	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Galium aparine	.	.	8	.	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	8	40	60
Galium verum	.	.	.	.	84	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	20	.	.	.	.	.	.	.	.	.	.
Geranium pratense	.	.	.	.	.	.	.	.	40	.	.	.	28	.	.	.	.	.	.	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Glechoma hederacea	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	100	.	.	.	.	.	.	.

Table A.4 (continued)

Heracleum sphondylium	.	.	.	.	.	12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	32	40
Lathyrus pratensis	.	.	8	.	.	12	44	48	48	8	24	16	60	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	16	.
Leucanthemum vulgare	.	.	.	.	.	.	.	.	.	.	.	.	16	.	.	20	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Lotus corniculatus	.	4	.	.	.	.	.	28	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Lotus pedunculatus	.	.	.	.	.	.	.	.	.	.	.	.	36	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oxalis acetosella	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	8	20	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Pinus sp.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	8	.	.	.	.
Plantago lanceolata	16	48	.	.	.	.	.	.	100	.	.	.	16	.	.	64	24	32	.	.	48	64	.	.	.	.	.	.	.	.	28	32	.	.	24	.
Polygala vulgaris	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	8	.	.	.	.
Potentilla erecta	.	24	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	48	12	.	.	.	.
Potentilla reptans	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	24	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Prunella vulgaris	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	16	44	.	32	.	.	.	.	.	.	.	.	.	.	.	20	.	.
Pteridium aquilinum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	24	8	.	.
Ranunculus acris	.	8	.	.	.	.	.	.	8	.	.	.	.	.	.	.	100	96	80	.	48	36	.	.	44	72	12	.	.	.	.	.	.	36	.	.
Rubus idaeus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	12	48	.	12	.	.	.	.	.	.	.	.	60	48
Rumex acetosa	.	.	.	.	.	.	24	.	.	.	.	.	.	.	.	.	16	28	.	.	.	.	8	.	.	.	16	.	20	.	20	12	.	16	.	.
Rumex obtusifolius	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	20	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Sanguisorba officinalis	.	.	.	.	.	.	.	.	.	.	64	16	.	20	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Scabiosa columbaria	.	.	.	.	28	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Senecio jacobea	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4	8	.	.	.	.	.	.	.	.	.	.	.	.
Stachys sylvatica	.	.	.	48	.	.	.	.	12	.	.	.	.	.	.	.	.	.	.	.	16	.	.	.	4	.	.	.	.	.	.	.	.	.	.	.
Stellaria holostea	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	12	.	.	.	.	4	.	.	.	.	.	.	20	.	.

Table A.4 (continued)

Trifolium pratense	.	.	.	.	.	.	.	.	.	.	.	44	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Trifolium repens	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	20	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Tussilago farfara	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Urtica dioica	.	.	.	.	.	.	.	.	.	.	20	.	.	32	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Veronica chamaedrys	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	12	4	12	.	.	.	.	16	.	.	.
Viola riviniana	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Agrostis capillaris	.	.	.	.	.	.	.	.	.	.	48	20	36	.	.	.	.	.	.	.	.	.	48	12	.	.	.	.	72	100	.	.	80	40	.	.
Agrostis stolonifera	100	80	52	8	12	32	80	72	72	32	.	.	.	44	72	32	32	.	36	36	84	28	.	.	.	32	.	.	.	.	32	60	.	.	.	.
Arrhenatherum elatius	.	.	60	76	24	48	32	68	.	72	84	100	32	80	80	36	.	.	.	.	8	.	.	.	.	.	.	.	80	100	.	.	72	80	.	.
Carex sp.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	8	.	.	.	.	.
Cynosurus cristatus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	20	.	.	.	44	24	.	.	.	.
Dactylis glomerata	.	.	.	.	.	.	44	.	.	.	.	.	.	20	.	12	.	.	.	.	40	.	.	.	.	.	.	.	.	16	.	.	.	12	.	.
Deschampsia cespitosa	.	.	20	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	20	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Festuca sp.	100	64	20	8	.	.	.	.	44	60	100	32	40	60	100	100	48	72	92	44	16	24	80	8	.	16	20	.	.	.	100	100	.	.	24	48
Holcus lanatus	84	60	24	32	44	12	20	8	32	68	.	40	16	.	20	.	44	72	.	16	24	12	64	36	.	.	48	80	16	.	20	48	48	24	16	.
Juncus conglomeratus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	16	.	.	.	.	.	.	.	.	.	.
Lolium perenne	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	32	.	.	.	.	.	.	.
Phleum bertolonii	.	.	.	.	.	.	24	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Poa pratensis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	60	52	.	.	.	.	.	32	.	.	.	.	.	.	.	.	.	.

Note. Abundance recorded as percent frequency of shoots in each 50cm × 50 cm quadrat. Site locations are given in Table 1.3.1.

Appendix B

Research paper published using data from this thesis:

Isolation of polymorphic microsatellites in the stemless thistle (*Cirsium acaule)* and their utility in other *Cirsium* species

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PRIMER NOTE

Isolation of polymorphic microsatellites in the stemless thistle (*Cirsium acaule)* and their utility in other *Cirsium* species

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Abstract

The genus *Cirsium* includes species with both widespread and restricted geographical distributions, several of which are serious weeds. Nine polymorphic microsatellite loci were isolated from the stemless thistle *Cirsium acaule*. Eight were polymorphic in *C. acaule*, six in *C. arvense* and seven in *C. heterophyllum*. One locus monomorphic in *C. acaule* showed polymorphism in *C. heterophyllum*. The mean number of alleles per locus was 4.1 in *C. acaule*, 6.2 in *C. arvense* and 2.9 in *C. heterophyllum*. These nine loci were also amplified in *C. eriophorum* and *C. vulgare*, suggesting that these markers may be of use throughout the genus.

Keywords: Asteraceae, *Cirsium*, microsatellite, thistle, weed, cross-species amplification

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The genus *Cirsium* occurs throughout the Northern Hemisphere with approximately sixty species native to Europe. *Cirsium arvense* and *C. vulgare* have become serious weeds throughout temperate zones across the globe. Several species reach geographical limits within the British Isles (*C. acaule, C. dissectum, C. eriophorum* and *C. heterophyllum*). We developed microsatellite markers in *Cirsium* towards a study investigating differences in population genetic structure between core and peripheral regions of a species' geographic range.

DNA extracted from fresh *Cirsium acaule* leaves following the protocol of Doyle and Doyle (1987) was used to create a genomic library enriched for the dinucleotide sequences $[GC]_n$, $[AC]_n$, $[GA]_n$ and $[GT]_n$, and the trinucleotide sequences $[CAA]_n$, $[GCC]_n$, $[CTG]_n$ and $[CAG]_n$. The protocol was essentially that of Edwards *et al.* (1996). In brief, 200ng DNA was digested with *Rsa*I (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK), ligated to *Mlu*I linkers (Edwards *et al.* 1996) and amplified by the polymerase chain reaction (PCR) using one linker sequence as a primer. The denatured fraction was hybridised with the dinucleotide and trinucleotide target sequences bound in combination (10 ng per oligonucleotide, one filter for trinucleotides, one for dinucleotides) to 0.5 cm^2 pieces of nylon membrane (Hybond N+, Amersham Pharmacia Biotech). After extensive washing, the bound *C. acaule* DNA was recovered and amplified as before. The linkers were removed by digestion with *Mlu*I (Amersham Pharmacia Biotech) and the DNA ligated into a pJV1 plasmid (a pUC19 plasmid containing a *BssH*II site, modified and supplied by K. Edwards, IACR, Long Ashton, UK). The plasmids were transformed into XL1 blue competent cells (Stratagene, La Jolla, CA, USA) and plated onto LB-agar plates containing ampicillin, IPTG and Xgal for selection. Colonies containing microsatellite sequences were identified by blotting onto nylon membrane and hybridising against the target sequences radiolabelled with ^{32}P-dCTP. Positive clones were sequenced in both directions using *Mlu*I linker sequence as primer and BIG DYE terminators on an ABI 377 Sequencer (Applied Biosystems, Foster City, CA, USA). PCR primers were designed from unique sequences with the assistance of Primer 3 (Rozen & Skaletsky 1996,1997). Primers from each pair were modified at the 5′end to include either a fluorescent phosphoramidite (TET, HEX or FAM) or the sequence GTTTCTT, added to reduce noise from variable adenylation during the PCR (Brownstein *et al.* 1996; Table 1).

Twenty-five individuals from each of three *Cirsium* species (*C. acaule, C. arvense* and *C. heterophyllum*) were used to assess polymorphism. PCR reactions were carried out in 10 μl volumes containing 50 ng genomic DNA, 1μM of each forward and reverse primer, 0.25 units Thermoprime Plus DNA polymerase (ABGene, Epsom, Surrey, UK) in the manufacturer's buffer (final concentrations 20mM $(NH_4)_2SO_4$, 75mM Tris-HCl pH9.0, 0.01% Tween), 1.5-2.5 mM $MgCl_2$ (see Table 1) and 200μM of each dNTP, with the addition of 0.5% Tween 20 (final concentration). PCR amplification was performed in a Hybaid Touchdown thermal cycler (Thermo Hybaid, Ashford, Middlesex, UK).

PCR products were initially visualised on 2% agarose gels stained with ethidium bromide; primer pairs amplifying a consistent product were then screened for polymorphism on 6% polyacrylamide gels stained with silver (Promega, Southampton, UK) (Bassam *et al.* 1991). Genotypes were assigned using an ABI 377 Sequencer and GeneScan v3.1 software (Applied Biosystems). Expected heterozygosities were calculated using Genetix v4.02 (Belkhir *et al.* 2001). Reaction profiles are given in Tables 1 and 2.

Of the 110 clones sequenced, 44 contained dinucleotide repeats, 3 contained trinucleotide repeats and 1 contained a tetranucleotide repeat. Primers were designed from 24 unique sequences with a minimum of 9 uninterrupted repeat units. Seven did not produce a product of the expected size and eight did not consistently amplify across all individuals of at least one species. All 24 sequences were submitted to the EMBL database (Accession numbers AJ457836-AJ457859). Eight loci detected polymorphism in *C. acaule*, 6 in *C. arvense* and 7 in *C. heterophyllum*. These revealed 2 - 7 alleles per locus with a mean observed heterozygosity of 0.60 in *C. acaule*, 0.74 in *C. arvense* and 0.41 in *C. heterophyllum* (Tables 1 and 2). PCR amplification of all nine loci was also observed in the more distant species *C. eriophorum* and *C. vulgare*; polymorphism in these species was not assessed (Table 2). A search of the EMBL sequence database identified a 47-bp region of similarity between sequence *Caca20* (AJ457855) and the NADH dehydrogenase gene from a range of plant species including *Arabidopsis thaliana* and *Oryza sativa*.

Microsatellite repeats are found less frequently in plants than animals (Lagercrantz *et al.* 1993). There is generally a lower level of cross-utility of loci among different plant taxa and when amplification occurs, fewer of the loci are usually found to be polymorphic (Whitton *et al.* 1997; Peakall *et al.* 1998). Screening loci across several congeneric species, as reported here, demonstrates that some loci that show poor amplification, or appear monomorphic in the species from which the markers are developed, may be of utility and detect polymorphism in related species. Cross-amplification of the *Cirsium acaule* markers in the other species described here suggests that these markers are likely to be of wider application within the genus *Cirsium*.

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Table 1 Characteristics of nine *Cirsium acaule* microsatellite loci

Locus (EMBL No)	Repeat in cloned allele	Modification at 5′ primer end (5′-3′)	Primer sequence (5′-3′)	Allele size range (bp)	Cycling conditions		No. alleles	H_o	H_e
					Program	$MgCl_2$ (mM)			
Caca01 (AJ457836)	$(CA)_{10}$	F: HEX- R: GTTTCTT-	TTT GAA GTG GAT CTT CGC ACG CAT GGG AGA CGA ACT AAC AGA TGC	239-233	64	2.5	4	0.64	0.63
Caca04 (AJ457839)	$(CA)_{12}$	F: GTTTCTT- R: FAM-	ATC ACC GCT TCC ACC GTC TC GCT TAT TAG AAC CGC CAT TGA AAG C	103-122	65/55TD	2.5	3	0.52	0.42
Caca05 (AJ457840)	$(CA)_{12}$	F: TET- R: TTTCTT-	ACC CAA CCC TCG ATC TGA A GAG GAT ACC GGC GAT TGT TA	164-166	62/52TD	1.5	2	0.52	0.52
Caca07 (AJ457842)	$(GT)_9(GA)_{11}$	F: GTTTCTT- R: HEX-	CCC AAA CTC CCA CCT TCA TTT G GTC GGA GAT GCT CCG GTG AC	148-160	64	2.5	5	0.72	0.71
*Caca10** (AJ457845)	$(TTC)_8$-$(TC)_{20}$	F: GTTTCTT- R: FAM-	GAA TTC GCG ACA ACA CAC GC GGT AAG GAA TGA ATG ATT GGG CTC	196	65/55TD	1.5	4	NCA	
Caca16 (AJ457851)	$(GT)_{10}$	F: TET- R: GTTTCTT-	TCG TGC TCT TCG ATT GAT TG CAG AAA ACC GCT CCA TTG C	117-135	60	2.5	4	0.48	0.52
*Caca17** (AJ457852)	$(CT)_{12}(GT)_9$-$(GT)_4$-$(GT)_8$	F: TET- R: GTTTCTT-	GGC ATA CTG ACA TTC TCA AAC GC CGT GAT GTG ATG GCA TGT TC	328-332	55	1.5	2	NCA	
*Caca22*** (AJ457856)	$(TC)_9$-$(TC)_9$	F: TET- R: GTTTCTT-	GGC TCT GCC TCA CCC ATC TC AGG TGT TCA GCA CGG TTC GG	190	65/55TD	1.5	1	0	0
Caca24 (AJ457858)	$(CA)_{10}$	F: HEX- R: GTTTCTT-	TGG ATA ACG CGC TAG ATC AC AAG AAC TCA ATT AGT AGG AAG TGG	232-244	62/52TD	2.5	7	0.71	0.73

PCR Programs

- 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)

Note: * Locus not amplifying consistently across all individuals (NCA) in *C. acaule* but of utility in *C. heterophyllum* and *C. arvense*; **monomorphic in *C. acaule* but polymorphic in *C. heterophyllum*. See Table 2. n = 25 individuals tested.

Table 2 Amplification and polymorphism of *Cirsium acaule* microsatellite loci in other *Cirsium* species

Locus	Cycling conditions		*Cirsium arvense* (*n* = 25)				*Cirsium heterophyllum* (*n* = 25)				*Cirsium vulgare* (*n* = 5)	*Cirsium eriophorum* (*n* = 5)
	Program	$MgCl_2$ (mM)	Allele size range (bp)	No. alleles	H_o	H_e	Allele size range (bp)	No. alleles	H_o	H_e		
Caca01	64	2.5	233-242	5	0.96	0.66	235-237	2	0.28	0.31	+	++
Caca04	65/55TD	2.5	103-119	5	0.60	0.55	112-126	3	0. 16	0.34	+	+
Caca05	62/52TD	1.5	144-174	7	0.92	0.72	144	1	0	0	++	++
Caca07	64	2.5	142-168	7	NCA		-	NP	0	0	+	+
Caca10	65/55TD	1.5	167-183	6	0.48	0.60	168-192	2	0.76	0.63	+	++
Caca16	60	2.5	-	NP	0	0	118-143	3	0.96	0.60	++	++
Caca17	55	1.5	290	1	0	0	305-317	3	0.12	0.11	+	+
Caca22	65/55TD	1.5	192-218	7	NCA		210-227	3	0.08	0.21	+	+
Caca24	62/52TD	2.5	220	1	0	0	222-266	4	0.48	0.53	++	++

Note: NP = no product of the expected size, NCA = not amplifying consistently across all individuals. + indicates PCR amplification observed, ++ indicates possible polymorphism. PCR programs are detailed in Table 1.

Appendix C

Abundance and quantitative trait summary data for
Cirsium acaule, C. arvense, C. eriophorum and C. heterophyllum
populations

Table C.1 Summary data for *C.acaule* populations (mean + standard error)

Code	Latitude	Abundance (*clumps/m²*) + SE		Clump Diameter (cm) + *SE*		Leaf morphology (cm) Length (l) + *SE*		Width (w) + *SE*		l:w + *SE*		Seed mass per capitulum (g) + *SE*		% Failing to set seed
AD1	50.587	0.52	*0.04*	35.50	*3.76*	12.00	*0.66*	2.67	*0.13*	4.56	*0.23*	0.0592	*0.0116*	6.7
AD2	50.672	0.68	*0.00*	26.80	*3.06*	9.73	*0.45*	2.57	*0.16*	3.92	*0.22*	0.0898	*0.0125*	0.0
AD3	50.630	0.38	*0.06*	25.50	*2.73*	11.40	*0.45*	2.23	*0.14*	5.29	*0.27*	0.0700	*0.0069*	3.3
AW1	51.209	0.22	*0.06*	32.50	*4.10*	11.47	*0.45*	2.57	*0.20*	4.75	*0.30*	0.0897	*0.0146*	16.7
AW2	51.262	0.54	*0.02*	31.80	*2.65*	9.77	*0.47*	2.53	*0.17*	4.00	*0.21*	0.0651	*0.0118*	10.0
AW3	51.269	1.50	*0.10*	29.00	*1.94*	10.00	*0.59*	2.67	*0.14*	3.78	*0.15*	0.0625	*0.0117*	33.3
AB1	51.447	0.64	*0.12*	51.00	*2.96*	12.83	*0.35*	2.73	*0.13*	4.79	*0.18*	0.1077	*0.0193*	26.7
AB2	51.430	0.36	*0.04*	30.40	*3.54*	12.40	*0.54*	2.60	*0.16*	4.95	*0.32*	0.0343	*0.0087*	30.0
AB3	51.327	0.50	*0.06*	38.00	*3.82*	12.27	*0.67*	2.60	*0.12*	4.74	*0.22*	0.0440	*0.0060*	10.0
AG1	51.842	1.04	*0.08*	37.90	*3.39*	9.20	*0.19*	2.20	*0.07*	4.22	*0.12*	0.0436	*0.0108*	33.3
AG2	51.865	0.88	*0.00*	34.10	*4.38*	10.43	*0.42*	2.43	*0.13*	4.49	*0.37*	0.0576	*0.0136*	26.7
AG3	51.842	1.28	*0.32*	39.50	*2.52*	10.40	*0.39*	2.17	*0.14*	4.98	*0.23*	0.0514	*0.0090*	13.3
AP1	53.262	0.32	*0.04*	48.00	*5.38*	11.57	*0.42*	2.77	*0.12*	4.28	*0.24*	0.0403	*0.0080*	33.3
AP2	53.138	1.16	*0.08*	48.50	*5.97*	12.63	*0.73*	2.97	*0.17*	4.34	*0.23*	0.0209	*0.0064*	53.3

Table C.2 Summary data for *C.arvense* populations (mean + standard error)

Code	Latitude	Abundance (shoots/m^2)	*SE*	Leaf morphology (cm) Length (l)	*SE*	Width (w)	*SE*	l:w	*SE*	Height (cm)	*SE*	Flowers per plant	*SE*	Seed mass per capitulum (g)	*SE*	% Failing to set seed
RD1	50.677	20.00	*2.00*	11.17	*0.34*	4.50	*0.16*	2.51	*0.10*	105.0	*3.3*	36.3	*3.9*	0.0831	*0.0077*	0.0
RD2	50.684	21.00	*3.00*	10.63	*0.53*	4.67	*0.24*	2.31	*0.10*	115.7	*5.1*	25.8	*3.2*	0.0463	*0.0064*	0.0
RB1	51.439	26.00	*3.00*	10.00	*0.54*	4.00	*0.21*	2.54	*0.13*	85.5	*3.2*	19.3	*2.5*	0.0492	*0.0058*	0.0
RB2	51.430	26.00	*6.00*	11.40	*0.48*	4.23	*0.15*	2.70	*0.08*	109.5	*4.0*	30.7	*3.2*	0.0397	*0.0083*	0.0
RG1	51.839	31.50	*3.50*	8.03	*0.36*	3.50	*0.19*	2.34	*0.10*	75.9	*4.1*	11.3	*1.9*	0.0573	*0.0055*	0.0
RG2	51.862	37.00	*1.00*	10.33	*0.52*	4.20	*0.36*	2.88	*0.45*	78.7	*3.9*	18.4	*1.7*	0.0154	*0.0048*	6.6
RP1	53.214	33.00	*1.00*	9.57	*0.37*	4.47	*0.20*	2.17	*0.08*	103.0	*2.3*	25.8	*1.6*	0.1062	*0.0078*	0.0
RP2	53.145	29.50	*2.50*	11.13	*0.36*	4.80	*0.23*	2.35	*0.07*	127.7	*3.4*	29.9	*2.9*	0.0392	*0.0048*	0.0
RC1	54.521	61.00	*1.00*	11.67	*0.41*	4.03	*0.19*	2.95	*0.13*	109.5	*1.7*	28.6	*3.3*	0.0542	*0.0056*	0.0
RC2	54.527	46.50	*2.50*	14.67	*0.50*	5.43	*0.22*	2.72	*0.07*	115.7	*4.4*	45.3	*4.8*	0.0591	*0.0076*	0.0
RS1	56.389	24.50	*2.00*	12.40	*0.38*	4.90	*0.21*	2.51	*0.10*	107.2	*5.2*	32.4	*6.1*	0.0740	*0.0070*	0.0
RS2	56.364	30.00	*3.50*	10.50	*0.45*	4.32	*0.19*	2.44	*0.13*	121.4	*3.7*	27.6	*5.4*	0.0184	*0.0029*	0.0
RM1	57.101	25.50	*2.50*	13.83	*0.58*	4.50	*0.36*	3.21	*0.15*	153.3	*6.9*	53.5	*7.4*	0.0160	*0.0018*	0.0
RM2	57.005	39.50	*5.50*	12.17	*0.47*	4.43	*0.21*	2.78	*0.09*	119.7	*4.7*	19.9	*2.1*	0.0303	*0.0040*	0.0

Table C.3 Summary data for *C.eriophorum* populations (mean + standard error)

Code	Latitude	Abundance (shoots/m^2)	+ *SE*	Leaf morphology (cm) Length (l)	+ *SE*	Width (w)	+ *SE*	l:w	+ *SE*	Height (cm)	+ *SE*	Flowers per plant	+ *SE*	Seed mass* per capitulum (g)	+ *SE*	% Failing to set seed
ED1	50.677	0.36	*0.04*	51.27	*4.17*	14.60	*0.94*	3.55	*0.27*	87.2	*3.8*	15.9	*3.7*	**	**	**
EW1	51.209	0.96	*0.12*	63.80	*1.19*	16.73	*0.57*	3.87	*0.13*	118.7	*3.2*	10.0	*1.2*	2.0230	*0.1933*	5
EW2	51.259	0.40	*0.00*	72.80	*2.57*	21.53	*1.44*	3.50	*0.17*	129.3	*4.2*	18.3	*2.4*	1.6140	*0.1644*	0
EW3	51.202	1.84	*0.12*	74.47	*2.37*	15.80	*1.10*	4.97	*0.30*	158.0	*4.2*	19.6	*2.5*	1.7670	*0.2050*	0
EB1	51.441	0.68	*0.28*	75.00	*2.07*	17.13	*0.68*	4.44	*0.14*	131.7	*2.4*	11.5	*1.7*	0.6745	*0.1310*	10
EB2	51.430	0.76	*0.12*	56.67	*1.69*	15.60	*0.75*	3.73	*0.19*	104.7	*5.4*	14.3	*1.9*	0.6475	*0.1130*	5
EB3	51.420	0.40	*0.08*	52.33	*2.38*	11.80	*0.55*	4.48	*0.15*	122.7	*5.0*	15.9	*2.3*	0.6295	*0.1654*	5
EG1	51.847	0.94	*0.22*	53.73	*1.93*	14.07	*0.43*	3.85	*0.15*	96.9	*3.9*	5.2	*0.4*	0.6835	*0.1532*	15
EG2	51.865	0.67	*0.24*	85.40	*3.34*	20.80	*1.07*	4.15	*0.14*	157.0	*5.4*	7.7	*0.5*	2.5815	*0.1987*	0
EG3	51.842	0.90	*0.10*	81.20	*2.15*	19.30	*0.61*	4.29	*0.19*	122.7	*5.0*	16.3	*2.5*	1.1945	*0.1703*	0
EP1	53.256	0.78	*0.34*	58.53	*2.05*	15.40	*0.86*	3.88	*0.12*	131.3	*7.7*	8.4	*0.9*	2.1750	*0.2410*	0
EP2	53.258	0.56	*0.00*	55.47	*1.88*	14.40	*0.79*	3.97	*0.17*	111.9	*5.1*	11.1	*1.4*	1.5730	*0.1694*	5
EP3	53.258	2.34	*0.34*	55.13	*1.71*	11.47	*0.62*	4.91	*0.18*	150.7	*6.3*	12.2	*1.5*	2.2220	*0.2267*	0
EY1	54.257	0.30	*0.06*	72.87	*2.51*	16.13	*0.76*	4.57	*0.14*	139.1	*4.5*	7.3	*1.0*	1.8260	*0.1322*	0

NOTES: *Adjusted for seed predation (see methods). **Plants were destroyed before seed could be collected.

Table C.4 Summary data for *C.heterophyllum* populations (mean + standard error)

Code	Latitude	Abundance (shoots/m^2)	+ *SE*	Leaf size (cm) Length (l)	+ *SE*	Width (w)	+ *SE*	l:w	+ *SE*	Height (cm)	+ *SE*	Flowers per plant	+ *SE*	Seed mass per capitulum (g)	+ *SE*	% Failing to set seed
HP1	53.214	41.0	*8.0*	46.1	*2.08*	8.63	*0.41*	5.42	*0.24*	82.0	*2.2*	2.5	*0.3*	0.0017	*0.0010*	90.0
HP2	53.231	43.0	*3.0*	51.8	*2.02*	9.57	*0.35*	5.45	*0.19*	105.3	*4.2*	3.3	*0.4*	0.0321	*0.0129*	63.3
HP3	53.241	32.0	*6.0*	53.6	*1.46*	10.17	*0.34*	5.34	*0.22*	89.1	*3.7*	2.7	*0.3*	0.0003	*0.0003*	96.7
HP4	53.166	27.0	*7.0*	61.1	*2.83*	10.73	*0.54*	5.76	*0.21*	108.0	*4.6*	2.6	*0.2*	0.0022	*0.0018*	90.0
HC1	54.408	41.0	*5.0*	51.5	*1.56*	8.63	*0.39*	6.12	*0.30*	89.1	*2.7*	2.5	*0.4*	0.0102	*0.0025*	46.7
HC2	54.439	24.0	*3.0*	52.4	*2.52*	9.77	*0.36*	5.47	*0.33*	89.1	*3.1*	2.3	*0.3*	0.0008	*0.0004*	80.0
HC3	54.862	46.0	*10.0*	51.3	*2.52*	9.23	*0.46*	5.62	*0.21*	91.7	*2.8*	1.8	*0.2*	0.0176	*0.0053*	51.7
HC4	54.447	39.0	*0.0*	46.5	*1.84*	9.63	*0.39*	4.91	*0.24*	84.8	*3.2*	2.4	*0.2*	0.0101	*0.0035*	63.3
HS1	56.490	45.0	*0.0*	48.7	*1.39*	9.93	*0.49*	5.01	*0.19*	82.1	*3.9*	5.8	*0.7*	0.0348	*0.0075*	36.7
HS2	56.400	32.5	*5.5*	46.3	*2.21*	8.37	*0.29*	5.55	*0.20*	96.7	*3.4*	1.7	*0.2*	0.0709	*0.0126*	25.9
HS3	56.321	53.0	*4.0*	58.8	*2.48*	10.60	*0.46*	5.60	*0.20*	100.4	*3.7*	3.5	*0.5*	0.0620	*0.0098*	16.7
HM1	57.101	53.5	*6.5*	40.1	*1.67*	9.07	*0.27*	4.46	*0.19*	97.1	*3.3*	4.9	*0.6*	0.2121	*0.0180*	6.7
HM2	57.015	72.0	*1.0*	54.8	*2.56*	9.17	*0.42*	6.07	*0.32*	104.1	*3.0*	3.0	*0.2*	0.2129	*0.0223*	6.7
HM3	57.327	72.0	*4.0*	53.2	*1.25*	10.00	*0.33*	5.38	*0.17*	103.3	*2.4*	2.3	*0.2*	***	***	***
HM4	57.420	70.5	*2.5*	59.7	*1.66*	9.03	*0.43*	6.75	*0.25*	86.9	*2.9*	2.4	*0.3*	0.1011	*0.0131*	0.0
HN1	57.990	51.5	*1.5*	52.7	*1.11*	10.63	*0.49*	5.08	*0.21*	94.5	*4.8*	2.9	*0.3*	***	***	***
HN2	58.243	41.0	*1.0*	63.7	*2.02*	7.83	*0.34*	8.32	*0.40*	107.6	*3.1*	2.7	*0.2*	0.0533	*0.0077*	3.4
HN3	57.753	44.5	*3.5*	67.9	*3.15*	12.40	*0.51*	5.55	*0.29*	111.5	*6.6*	3.6	*0.3*	0.1626	*0.0181*	3.3

NOTES: ***Capitula were unripe and therefore not collected.