
Article:
Jump, A.S., Woodward, I. and Burke, T. (2003) Cirsium species show disparity in patterns of genetic variation at their range-edge, despite similar patterns of reproduction and isolation. New Phytologist, 160 (2). pp. 359-370. ISSN 0028-646X

https://doi.org/10.1046/j.1469-8137.2003.00874.x

New Phytologist

Research

Cirsium species show disparity in patterns of genetic variation at their range-edge, despite similar patterns of reproduction and isolation

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Received: *25 April 2003*
Accepted: *23 June 2003*

doi: 10.1046/j.1469-8137.2003.00874.x

Summary

• Genetic variation was assessed across the UK geographical range of *Cirsium acaule* and *Cirsium heterophyllum*. A decline in genetic diversity and increase in population divergence approaching the range edge of these species was predicted based on parallel declines in population density and seed production reported seperately. Patterns were compared with UK populations of the widespread *Cirsium arvense*.

• Populations were sampled along a latitudinal transect in the UK and genetic variation assessed using microsatellite markers.

• *Cirsium acaule* shows strong isolation by distance, a significant decline in diversity and an increase in divergence among range-edge populations. Geographical structure is also evident in *C. arvense*, whereas no such patterns are seen in *C. heterophyllum.*

• There is a major disparity between patterns of genetic variation in *C. acaule* and *C. heterophyllum* despite very similar patterns in seed production and population isolation in these species. This suggests it may be misleading to make assumptions about the geographical structure of genetic variation within species based solely on the present-day reproduction and distribution of populations.

Key words: *Cirsium*, microsatellites, geographical range, genetic variation, isolation by distance, clonal reproduction.

Introduction

In a campanion paper (Jump & Woodward, 2003) we report variation in demographic parameters throughout the UK latitudinal range of three *Cirsium* species. *Cirsium acaule* (stemless thistle) reaches a northern range limit and *Cirsium heterophyllum* (melancholy thistle) reaches a southern range limit in the central region of the UK. Both species display a decline in seed production toward their UK range edge. At its northern range edge, *C. acaule* produces 37% of the maximum seed mass recorded in its core region, at the southern range edge of *C. heterophyllum*, seed production is only 1.2% of maximum. Both species also show a decline in the density of populations approaching the range edge, indicating that peripheral populations are more geographically isolated from one another than populations in core areas of the range. A third species, *C. arvense* (creeping thistle), which is widespread throughout the UK and therefore does not reach a range limit, shows no latitudinal pattern in either of these traits.

One of the consequences of declining population density approaching the range edge is that populations become increasingly isolated, both from populations further toward the core of the species range and from each other (Brown, 1984). As geographical isolation increases, a reduction in both seed dispersal and pollen flow will result in decreased gene flow between populations (Ellstrand & Hoffman, 1990; van Dorp *et al.*, 1996). The resulting genetic isolation may lead to pronounced geographical structuring in genetic variation within a species as population differentiation increases (Lesica & Allendorf, 1995). Both genetic drift and inbreeding are likely to be of increased importance in isolated populations, with the result that genetic diversity may be reduced toward the species periphery (Barrett & Kohn, 1991; Ellstrand &

Elam, 1993; Raijmann *et al.*, 1994; Schaal & Leverich, 1996; Lammi *et al.*, 1999).

Seed production declines approaching the periphery of *C. acaule* and *C. heterophyllum*, both in terms of the proportion of each population that produces seed and the amount of seed produced in each capitulum. Declining seed production (Pigott & Huntley, 1981; Reinartz, 1984; Eckert & Barrett, 1993; García *et al.*, 2000; Dorken & Eckert, 2001) and increased seed abortion (García *et al.*, 2000) have been reported approaching the periphery of many species. It is possible that genetic diversity within these peripheral populations may be severely reduced as a consequence of a small subsample of the flowering population being responsible for any establishment from seed. Poor seed production and increased geographical isolation may interact, resulting in demographic instability in peripheral populations (Schaal & Leverich, 1996) with the potential to induce genetic bottlenecks at the periphery (Lesica & Allendorf, 1995). Such genetic subsampling effects are likely to exacerbate the loss of diversity through the processes outlined above.

There has been considerable theoretical investigation of the evolutionary limits to a species range (Bradshaw, 1991; Hoffman & Blows, 1994; Kirkpatrick & Barton, 1997; Barton, 2001). Although this work does not set out to assess these evolutionary hypotheses directly, it has the potential to inform on some aspects of theory regarding the divergence and diversity of range edge populations. For example, it is commonly assumed that isolation and reduced size of peripheral populations will lead to a reduction in their genetic diversity (Ellstrand & Elam, 1993; Schaal & Leverich, 1996) and possibly a reduction in the likelihood that they might adapt to conditions beyond the range edge (Bradshaw, 1991). However, it has been hypothesized that fluctuating environmental conditions in peripheral areas might maintain more genotypes here if selection favours genetic flexibility, whereas relatively more stable conditions in core areas may favour the high average fitness of only a few genotypes (Safriel *et al.*, 1994). This would potentially lead to lower diversity of populations in core rather than peripheral regions of a species' range. Furthermore, although genetic divergence of peripheral populations is predicted based on increased geographical isolation (Schaal & Leverich, 1996), it has also been suggested that reduced density of peripheral populations may render them likely to be swamped by gene flow from populations further toward the core. This would prevent their divergence and adaptation to local (range-edge) conditions, thereby restricting range expansion (Barton, 2001).

Considering the patterns in population density and seed production reported by Jump & Woodward (2003), the aims of this study were to determine whether the declines in population density and reproductive potential approaching the range boundary of *C. acaule* and *C. heterophyllum* are reflected in the predicted parallel decline in genetic variability and increase in divergence of range-edge populations. To assess potential latitudinal patterns of diversity in these species that may occur irrespective of patterns in population density and reproduction, these traits were also assessed in the widespread *C. arvense* (which shows no latitudinal patterns in seed production or population density).

It is not the aim of this work to present a comprehensive study of the phylogeography of these species. Consequently, with the exception of *C. heterophyllum*, these species were sampled only within their UK range. Although *C. heterophyllum* reaches a southern lowland limit in the UK, it occurs at much higher altitudes throughout the mountains of Europe: thus, more southerly European populations exist beyond its southern lowland UK limit. Additional populations of *C. heterophyllum* from Switzerland and Italy have therefore been included in this study in an attempt to determine whether any potential decline in genetic variation toward the southern periphery of this species in the UK is a result of a range edge being reached. If this is so then it is expected that the genetic variation in this species' southern peripheral region in the UK should be lower than both that in its core lowland region in Scotland and core high-altitude regions in more southerly areas of Europe.

Materials and Methods

Sampling procedure

Populations were sampled along a latitudinal transect running the length of Scotland and England, with additional populations of *C. heterophyllum* sampled in the Swiss and Italian Alps (Fig. 1, Tables 1 and 2). Twenty-five individuals were sampled from each population, these were as spatially separated as possible given the area covered by the population. A 4-cm^2 leaf sample was taken from each individual, dried in silica gel in the field and stored in dry silica gel until analysed. 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.

Genotyping

Individuals were genotyped at microsatellite loci originally isolated in *C. acaule* by Jump *et al.* (2002). Microsatellites were amplified from leaf extract following a modified version of the protocol presented by Wang *et al.* (1993) and tested by Rogers *et al.* (1996).

A 0.5 cm^2 sample of dried leaf tissue was ground in 60 µl 0.5 M NaOH and centrifuged at 18 300 ***g*** for 5 min. Then, 15 µl of the supernatant was added to 485 µl sterile 100 mM Tris-HCl (pH 8) and mixed well. This extract was then used directly in each polymerase chain reaction (PCR) reaction. A 2 µl sample of leaf extract was amplified in a total volume of 15 µl containing 2 mg ml^{-1} bovine serum albumin (BSA)

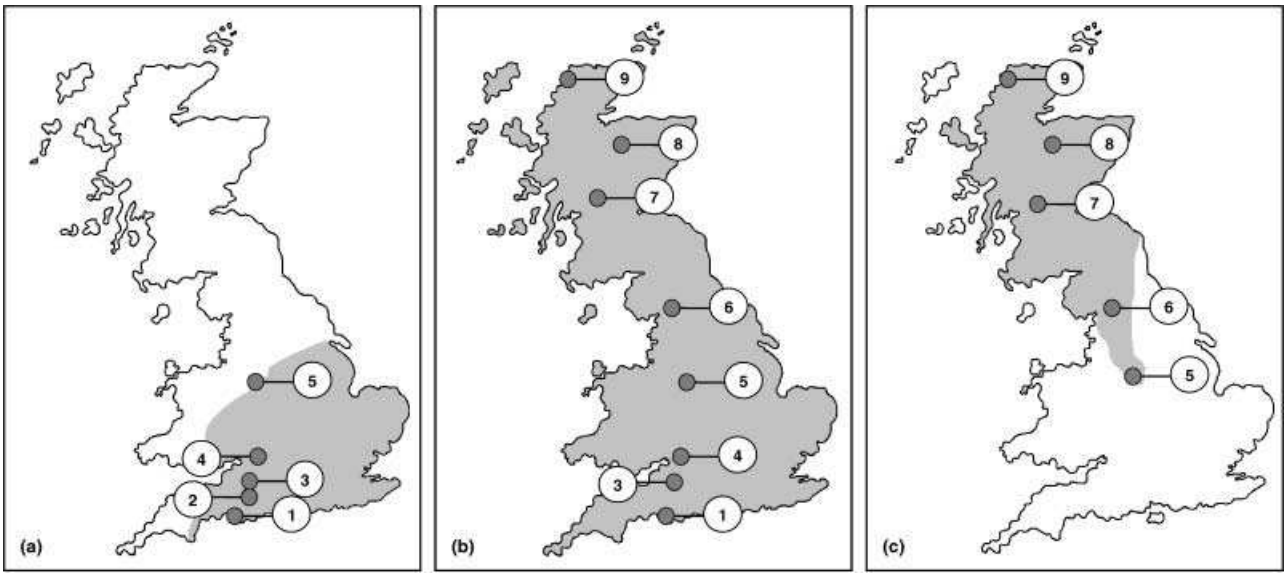

Fig. 1 The distribution of *Cirsium* species in England, Scotland and Wales (indicated by the grey shaded area) and approximate location of survey regions (dark grey circles): (a) *C. acaule*, (b) *C. arvense*, (c) *C. heterophyllum*. Four additional populations of *C. heterophyllum* were surveyed in the Swiss and Italian Alps. Numbers indicate the survey region (see Tables 1 and 2).

Table 1 Genetic diversity in *Cirsium acaule* populations

Population code	Location	Population area (m^2)	Allelic richness
11	50.587 N 2.032 W	73000	3.69 (0.30)
12	50.672 N 2.587 W	4000	3.88 (0.52)
13	50.630 N 1.969 W	3000	3.68 (0.50)
21	51.209 N 2.092 W	8000	4.00 (0.49)
22	51.262 N 2.034 W	2000	4.27 (0.46)
23	51.269 N 2.023 W	53000	3.69 (0.64)
31	51.447 N 2.401 W	8000	3.85 (0.55)
32	51.430 N 2.404 W	7000	3.72 (0.48)
33	51.327 N 2.791 W	7000	3.35 (0.53)
41	51.842 N 2.107 W	15000	3.63 (0.42)
42	51.868 N 2.073 W	4000	3.83 (0.44)
43	51.842 N 1.996 W	30000	3.85 (0.48)
51	53.262 N 1.733 W	6000	2.53 (0.19)
52	53.138 N 1.714 W	25000	2.87 (0.44)
Mean			3.63
SE			(0.12)

Allelic richness indicates mean allelic richness averaged over loci. Standard errors are given in parentheses. The first number of the population code indicates the survey region, as shown in Fig. 1.

(fraction V), 0.5% dimethylsulphoxide (DMSO), 1× manufacturer's PCR buffer (final concentrations; 20 mM $(NH_4)_2SO_4$, 75 mM Tris-HCl pH9.0, 0.01% Tween), 200 μM each dATP, dCTP, dGTP and dTTP, 1 μM each forward and reverse primer, 0.25 units Thermoprime Plus DNA polymerase (ABGene, Epsom, Surrey, UK) and 1.5 or 2.5 mM $MgCl_2$. BSA (fraction V) was added to PCR conditions as recommended by Möhlenhoff *et al.* (2001). The PCR was performed in 96-well plates in a Hybaid Touchdown Thermal Cycler (Thermo Hybaid, Ashford, Middlesex, UK). Each set of reactions included a negative (water) and positive (known genotype) control. PCR programs and $MgCl_2$ concentrations follow those reported by Jump *et al.* (2002). Products were analysed on 5% polyacrylamide gels using an ABI 377 Sequencer running GENESCAN v3.1.2 software (Applied Biosystems, Foster City, MA, USA). Genotypes were assigned using GENOTYPER v2.5 (Applied Biosystems). Twenty-five individuals from each of the populations detailed in Tables 1 and 2 were genotyped for the loci listed in Table 3.

Data analysis

For *C. arvense* and *C. heterophyllum*, statistics were calculated in two ways: (1) using each sampled plant (ramet level

Table 2 Genetic diversity in *Cirsium arvense* and *C. heterophyllum* populations

Population Code	Location	Population area (m^2)	Allelic richness		N° genets	Clone size	D	E
			Ramets	Genets				
Cirsium arvense								
12	50.677 N 2.655 W	750	4.58 (0.61)	2.71 (0.17)	12	2.1	0.89	0.90
13	50.594 N 2.034 W	480	1.75 (0.25)	1.75 (0.25)	2	12.5	0.08	0.00
31	51.439 N 2.401 W	1400	4.01 (0.55)	2.40 (0.18)	18	1.4	0.97	1.00
32	51.430 N 2.404 W	3000	3.98 (0.89)	2.61 (0.41)	8	3.1	0.75	0.75
41	51.839 N 2.107 W	1300	4.55 (1.07)	2.39 (0.39)	14	1.8	0.92	0.94
42	51.862 N 2.071 W	160	4.15 (0.76)	2.66 (0.37)	9	2.8	0.71	0.70
51	53.214 N 1.765 W	210	5.10 (0.66)	2.80 (0.22)	13	1.9	0.91	0.93
52	53.145 N 1.728 W	310	4.59 (0.47)	2.64 (0.13)	16	1.6	0.92	0.94
62	54.527 N 2.329 W	90	4.35 (0.74)	2.57 (0.28)	14	1.8	0.92	0.94
63	54.532 N 2.365 W	120	3.23 (0.50)	2.44 (0.23)	8	3.1	0.80	0.80
71	56.389 N 5.196 W	15	2.40 (0.18)	2.03 (0.11)	8	3.1	0.49	0.46
72	56.364 N 5.183 W	40	1.65 (0.25)	1.83 (0.17)	3	8.3	0.16	0.09
82	57.101 N 3.987 W	225	4.90 (0.36)	2.77 (0.09)	11	2.3	0.93	0.95
83	57.005 N 4.170 W	180	3.15 (0.20)	2.23 (0.07)	14	1.8	0.92	0.94
91	57.967 N 4.735 W	36	2.00 (0.00)	2.00 (0.00)	2	12.5	0.08	0.00
92	58.163 N 4.990 W	200	2.41 (0.41)	2.15 (0.23)	8	3.1	0.49	0.46
Mean			3.55	2.37	10.0	4.0	0.68	0.68
SE			(0.29)	(0.08)	(1.2)	(0.9)	(0.08)	(0.09)
Cirsium heterophyllum								
A1	46.100 N 7.950 E	35	2.72 (0.40)	1.92 (0.19)	21	1.2	0.98	0.98
A2	45.840 N 7.744 E	45	2.24 (0.20)	1.69 (0.15)	18	1.4	0.94	0.91
A3	45.836 N 7.746 E	2150	2.57 (0.38)	1.73 (0.18)	21	1.2	0.98	0.98
A4	45.944 N 7.733 E	1850	2.78 (0.44)	1.92 (0.22)	13	1.9	0.80	0.67
51	53.214 N 1.765 W	45	2.32 (0.32)	1.85 (0.08)	18	1.4	0.98	0.97
52	53.231 N 1.844 W	30	2.01 (0.14)	1.77 (0.11)	10	2.5	0.75	0.57
53	53.241 N 1.780 W	100	1.57 (0.20)	1.55 (0.19)	4	6.3	0.42	0.00
54	53.166 N 1.879 W	860	2.41 (0.23)	1.89 (0.11)	16	1.6	0.94	0.90
61	54.408 N 2.337 W	72	2.39 (0.35)	1.86 (0.21)	11	2.3	0.88	0.80
62	54.439 N 2.587 W	120	3.19 (0.27)	2.05 (0.15)	15	1.7	0.87	0.78
63	54.862 N 2.508 W	100	2.89 (0.26)	1.93 (0.17)	19	1.3	0.97	0.96
64	54.447 N 2.387 W	900	3.15 (0.38)	2.11 (0.14)	24	1.0	1.00	1.00
65	54.377 N 2.346 W	18	1.81 (0.30)	1.68 (0.23)	7	3.6	0.59	0.29
71	56.490 N 4.748 W	200	2.61 (0.26)	1.85 (0.13)	16	1.6	0.95	0.91
72	56.400 N 5.213 W	30	1.88 (0.16)	1.75 (0.14)	10	2.5	0.78	0.63
73	56.321 N 3.685 W	160	2.32 (0.33)	1.85 (0.17)	15	1.7	0.93	0.89
81	57.101 N 3.987 W	150	2.98 (0.17)	2.06 (0.13)	21	1.2	0.98	0.97
82	57.015 N 4.162 W	340	3.30 (0.33)	2.11 (0.16)	21	1.2	0.97	0.95
83	57.327 N 3.021 W	40	2.02 (0.39)	1.81 (0.25)	7	3.6	0.59	0.29
84	57.420 N 2.627 W	50	2.69 (0.49)	1.89 (0.16)	19	1.3	0.97	0.96
91	57.990 N 4.814 W	40	2.33 (0.52)	1.89 (0.20)	17	1.5	0.95	0.93
92	58.243 N 5.177 W	70	2.58 (0.31)	2.05 (0.10)	19	1.3	0.97	0.95
93	57.753 N 5.011 W	30	2.15 (0.22)	1.81 (0.09)	12	2.1	0.88	0.79
Mean			2.47	1.87	15.4	2.0	0.87	0.79
SE			(0.10)	(0.03)	(1.1)	(0.2)	(0.03)	(0.06)

Allelic richness indicates mean allelic richness averaged over loci. N° genets, the number of unique multilocus genotypes detected in each population; Clone size = *n* ramet/*n* genet; D, Simpson's diversity index; E, Fager's measure of sample evenness. Standard errors are given in parentheses. The first number of the population code indicates the survey region, as shown in Fig. 1. *Cirsium heterophyllum* populations A1–A4 were surveyed in the Swiss and Italian Alps. All other populations were surveyed in the UK.

analysis) and (2) after the removal of duplicate multilocus genotypes from within each population (genet-level analysis). These species were sampled within dense stands, therefore 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, but removing duplicate multilocus genotypes may result in the over-representation of rare alleles and the under-representation of common

Table 3 Polymorphic microsatellite loci used to genotype *Cirsium* species. For each species, the total number of alleles detected at each locus is given in parentheses below the locus name

Species	Loci						
C. acaule	*Caca01* (6)	*Caca04* (8)	*Caca05* (4)	*Caca07* (6)	*Caca16* (9)	*Caca24* (8)	
C. arvense	*Caca01* (9)	*Caca04* (8)	*Caca05* (19)	*Caca10* (10)			
C. heterophyllum	*Caca01* (4)	*Caca04* (10)	*Caca10* (10)	*Caca16* (6)	*Caca17* (5)	*Caca22* (6)	*Caca24* (9)

For each species, the total number of alleles detected at each locus is given in parentheses after the locus name.

alleles (Widén *et al.*, 1994). Calculating statistics based on 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 & Waterway, 1993; McLellan *et al.*, 1997; Ivey & Richards, 2001). Where both ramet and genet values are presented for any statistic in this paper, the data are presented as a range with the ramet value first. Duplicate multilocus genotypes were extremely rare within samples representing populations of *C. acaule* as in this species plants grow as distinct patches (presumed genets; Pigott, 1968) 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). Allelic richness is used as an estimate of the genetic diversity of populations in this paper as this measure allows direct comparison between populations of different sample size, since the value for each population is calculated based on the size (n) of the smallest population being considered (El Mousadik & Petit, 1996). To facilitate comparison between species, this measure was calculated based on the minimum number of complete multilocus genotypes occurring within any population across all species. Consequently, allelic richness was adjusted for a sample size of 10 diploid individuals per population for *C. acaule* and at the ramet level of analysis of *C. arvense* and *C. heterophyllum*. At the genet level of analysis of *C. arvense* and *C. heterophyllum*, allelic richness was adjusted for a sample size of two individuals (the number of genets detected in each population of *C. arvense* and *C. heterophyllum* is reported in Tables 1 and 2).

FSTAT was 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 randomized within populations; the number of permutations was determined by FSTAT (at the 5% nominal level: *C. acaule*, 1680 permutations; *C. arvense*, 1280 permutations; *C. heterophyllum*, 3220 permutations). Loci were considered to be in HWE if greater than 5% of randomized data sets resulted in fixation indices (F_{IS}; Weir & 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 & Waterway, 1993). This resulted in the exclusion of *C. arvense* populations 13, 72 and 91 from this analysis. In order to ensure loci were independent, a test for genotypic disequilibria between all pairs of loci over all samples was also performed in FSTAT.

Population differentiation over all populations was assessed based on randomizing 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). Ten-thousand randomizations 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 & Roose, 1987; Widén *et al.*, 1994). For *C. 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:

$$D = 1 - \Sigma[N_j(N_j - 1)/N(N - 1)]$$

(N_j is the number of shoots of the jth genotype; 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 & Waterway, 1993; Widén *et al.*, 1994; Vasseur, 2001). Fager's (1972) E was also calculated:

$$E = (D - D_{min})/(D_{max} - D_{min})$$

(D_{max} and D_{min} are calculated across all populations of the species being investigated; E describes the evenness of the distribution of genotypes within the population, like D it varies between 0 and 1.)

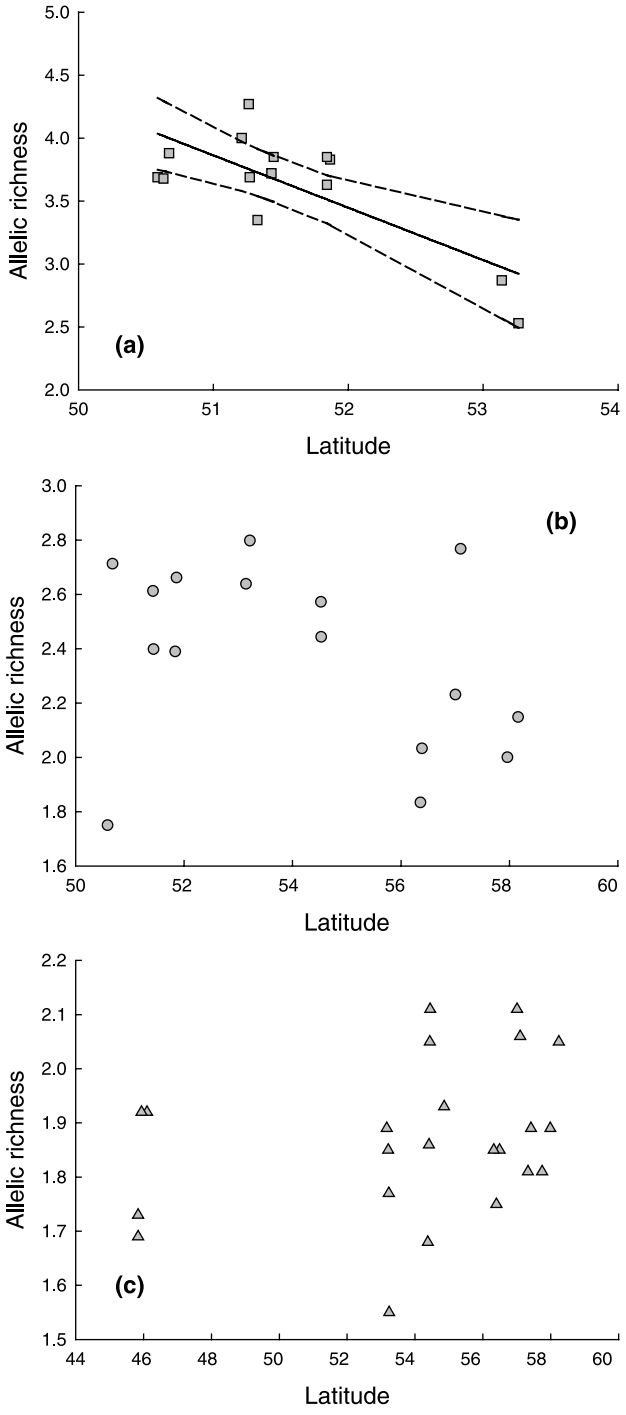

Fig. 2 Allelic richness in *Cirsium* populations as a function of latitude. (a) *Cirsium acaule*, regression: $y = 25.07 - 0.42x$, $R^2 = 0.57$, $P < 0.005$. (b) *Cirsium arvense* (genets). (c) *Cirsium heterophyllum* (genets). Dotted lines show 95% confidence limits of regression.

To investigate the effect of variation in population area (the surrogate for population size) on the analysis of latitudinal patterns in measures of genetic or clonal diversity, multiple regression analysis was performed in FSTAT. All diversity measures were regressed against population latitude and area; the significance of any relationship was assessed by a partial mantel test based on 10 000 randomizations of the data. There was no significant effect of population area on any diversity measure and it was therefore dropped from the model.

To determine whether diversity declines approaching the periphery of *C. acaule* and *C. heterophyllum* and if an underlying latitudinal pattern exists in *C. arvense*, allelic richness, clone size, D and E were regressed against population latitude using SIGMAPLOT 2001 for Windows v7 (SPSS Inc., Chicago, IL, USA). To estimate genetic distance between populations within each species and allow assessment of the genetic divergence of peripheral populations of *C. acaule* and *C. heterophyllum*, Nei's (1972) genetic distance was calculated for all possible pairs of populations and unrooted UPGMA trees produced using PHYLIP v3.6a (Felsenstein, 1989). PHYLIP was used to test the robustness of tree topologies: 1000 bootstrap replicates of the allele frequency data were generated in SEQBOOT and these were analysed in GENDIST. Tree topologies were created for all replicates using NEIGHBOUR and a consensus tree was generated in CONSENSE.

Genetic isolation by geographical distance

Isolation by distance was assessed using the programme IBD v1.2 (Bohonak, 2002) based on all combinations of untransformed data, log (genetic distance) and log (geographical distance). For *C. heterophyllum* the analyses were repeated following the removal of all non-UK populations from the data set. A Mantel test was performed using IBD on any correlation between geographical distance and genetic distance, based on 10 000 randomizations of the data. Confidence limits of any relationship were based on 10 000 bootstrap re-samples of the data.

Results

Genetic diversity within populations

Only *C. acaule* showed a significant relationship between genetic diversity and latitude. In *C. acaule*, allelic richness decreased with increasing latitude ($R^2 = 0.57$, $P < 0.005$, Fig. 2a). Maximum allelic richness in *C. acaule* was found to be 4.27 in the core area of its UK distribution, declining to a maximum of 2.87 in peripheral populations.

No relationship between genetic diversity and latitude was seen in either *C. arvense* or *C. heterophyllum* at either the ramet or genet level of analysis (Fig. 2b,c; ramet data not shown). Allelic richness is generally lower when populations are analysed at the genet level rather than the ramet level as a consequence of the reduction in minimum sample size inherent in calculating the genet-level estimate of this measure. There was no relationship with latitude in clonal diversity (D), evenness

(E), or clone size in either *C. arvense* or *C. heterophyllum* (Table 2).

Population genetic structure

Departure from HWE was not consistent across loci; however, many populations departed from HWE through either excess heterozygotes or homozygotes. In *C. acaule*, one population (7% of the total number of populations sampled) contained a significant excess of heterozygotes (negative F_{IS}). 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* showed an excess of heterozygotes whereas 13% showed an excess of homozygotes (positive F_{IS}). 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 showed an excess of heterozygotes whereas 13% showed an excess of homozygotes. There were no significant genotypic disequilibria between loci in *C. acaule* ($P > 0.05$). Genotypic disequilibria were detected in *C. arvense* and *C. heterophyllum*, although the loci involved were not consistent either between species or within species between the ramet and genet level analyses (data not shown).

Diversity within *Cirsium* species

Both total diversity (H_T) and the proportion of genetic diversity within populations (H_S) were high for all species (Table 4). The similarity of H_T values for *C. acaule* (0.643) and *C. heterophyllum* (0.639–0.647) indicates broadly comparable levels of genetic variability detected within these species; H_T was highest in *C. arvense* (0.715–0.751). Population differentiation was particularly high in *C. heterophyllum* (G_{ST} = 0.359–0.318) and *C. arvense* (0.246–0.131), but only moderate (0.066) in *C. acaule* (see Balloux & Lugon-Moulin, 2002 for discussion of population differentiation). 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 ($P < 0.001$).

Genetic distance and geographical structure

Mean Nei's (1972) genetic distance among population pairs was 0.147 (range 0.034–0.440) in *C. acaule*. In *C. arvense*, ramets it was 0.553 (range 0.121–1.458) and in genets it was 0.490 (range 0.107–1.264). Mean genetic distance between *C. heterophyllum* population pairs was 0.555 (range 0.070–1.460) for ramets and 0.533 (range 0.089–1.390) for genets. Unrooted tree diagrams representing Nei's (1972) genetic distance in each species are shown in Fig. 3.

Cirsium acaule populations formed a relatively tight cluster with two populations identified as outliers (populations 51 and 52, Fig. 3a). In *C. acaule*, the outliers indicated by genetic distance represent those populations that are found at the edge of the species geographical range. Bootstrap support for the separation of clusters of core and peripheral populations is 74%. The genetic distance between populations 51 and 52

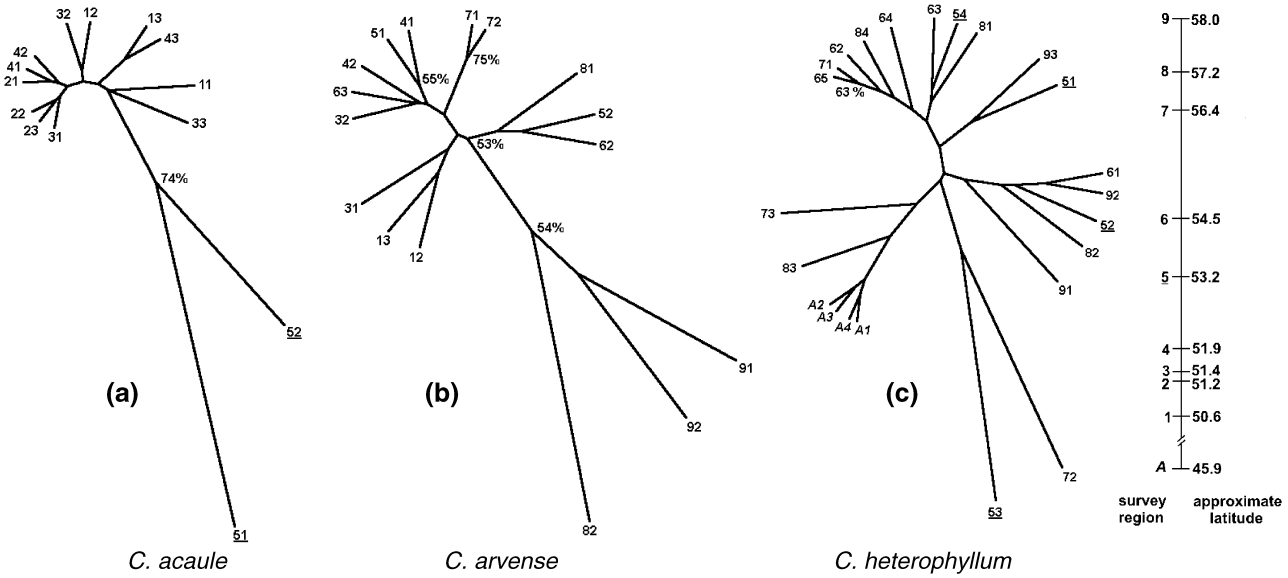

Fig. 3 Unrooted tree from UPGMA cluster analysis based on Nei's (1972) genetic distance between (a) *Cirsium acaule*, (b) *Cirsium arvense* and (c) *Cirsium heterophyllum* populations. Branch lengths are scaled relative to the maximum genetic distance between populations (*C. acaule*, 0.440; *C. arvense*, 1.264; *C. heterophyllum*, 1.390). Genet data only are shown for *C. arvense* and *C. heterophyllum*. The first number or letter of each site code indicates the survey region (see key). Populations from the edge of the geographic range of *C. acaule* and *C. heterophyllum* are underlined; *C. heterophyllum* populations surveyed in the Swiss and Italian Alps are in italics. Accurate site locations are given in Tables 1 and 2. Bootstrap values above 50% are placed at the nodes. Bootstrap values are derived from consensus trees and represent the percentage of 1000 trees where populations beyond the node grouped together.

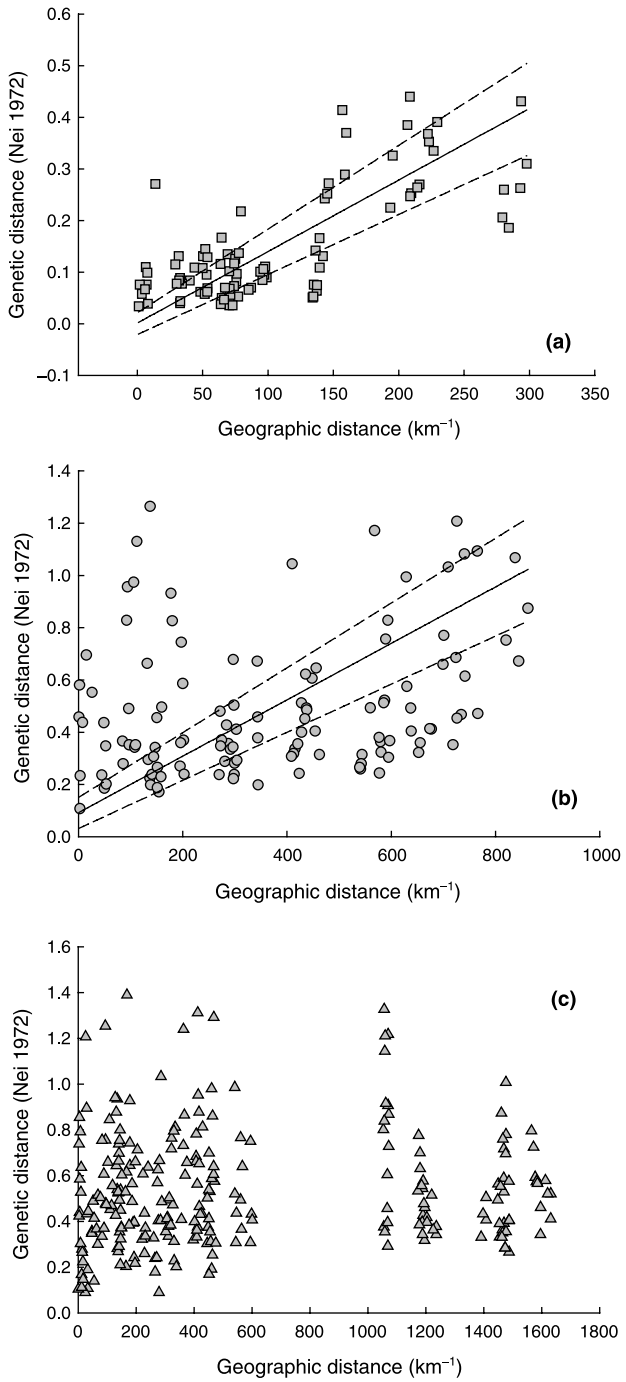

Fig. 4 Genetic distance (Nei, 1972) between populations as a function of geographic distance: (a) *Cirsium acaule* ($y = 1.35 \times 10^{-3} + 1.39 \times 10^{-3}x$, $R^2 = 0.56$, $P < 0.005$); (b) *Cirsium arvense* (genets: $y = 0.92 + 1.08 \times 10^{-3}x$, $R^2 = 0.09$, $P < 0.005$); (c) *Cirsium heterophyllum* (genets, all populations). Dotted lines show 95% confidence limits of regression.

was 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 was 0.297 (70%). This contrasts with a mean genetic distance of 0.088 (19%) between core populations.

Cirsium arvense does not reach a geographical limit within the UK. The clustering of populations was somewhat looser at the genet level when compared with the ramet level of analysis (Fig. 3b, ramet data not shown). At the genet level of analysis, the tree structure corresponds broadly with the geographical areas of the UK within which the populations were sampled. However, there is little bootstrap support for the clustering of *C. arvense* populations by region. Only at the genet level of analysis does the outlier group include two populations surveyed from the same latitude (populations 91 and 92); the mean genetic distance between outlying populations was 0.552 (44% of the maximum genetic distance recorded for this species). The mean genetic distance between outlying populations and any of the populations in other areas of the species UK range was 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*, outlying populations were relatively less divergent both from each other and other *C. arvense* populations when compared with the peripheral populations of *C. acaule* (Fig. 3a).

In *C. heterophyllum*, little geographical structuring of the tree is seen. Populations from the Swiss and Italian Alps cluster within the tree but this pattern is not seen with populations surveyed within any other broad geographical area in the UK. Regional clustering of *C. heterophyllum* populations is not supported by bootstrap values. 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 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 geographical range (Fig. 3c, ramet figure not shown).

A significant correlation between genetic distance and geographical distance was seen in *C. acaule* (Fig. 4a: $R^2 = 0.56$, $P < 0.005$) and *C. arvense* (ramets, $R^2 = 0.14$, $P < 0.0001$; genets, $R^2 = 0.09$, $P < 0.005$: Fig. 4b, ramet data not shown). No correlation between genetic distance and geographical distance (untransformed or log-transformed) was seen in *C. heterophyllum* (ramets or genets) either when the analysis included all populations surveyed or only those occurring in the UK (Fig. 4c, ramet data not shown).

Discussion

Diversity within *Cirsium* species

Reviews of allozyme diversity in plant species have been published by Loveless & Hamrick (1984) and Hamrick & Godt (1996). Although allozymes generally show a lower level of variability than microsatellites (Hedrick, 1999; Ouborg

Table 4 Genetic diversity averaged over all loci in *Cirsium* species

	Ramets			Genets		
Species	H_T	H_S	G_{ST}	H_T	H_S	G_{ST}
C. acaule	–	–	–	0.643 (0.044)	0.600 (0.042)	0.066 (0.012)
C. arvense	0.715 (0.086)	0.539 (0.058)	0.246 (0.015)	0.751 (0.067)	0.653 (0.047)	0.131 (0.026)
C. heterophyllum	0.639 (0.047)	0.410 (0.035)	0.359 (0.045)	0.647 (0.045)	0.441 (0.033)	0.318 (0.043)

H_T, Total gene diversity; H_S, gene diversity within populations; G_{ST}, among-population differentiation. Estimates calculated according to Nei (1987). For genet-level analysis of *C. arvense*, only populations containing four or more genets were analysed (see Table 2). Standard errors are given in parentheses.

Table 5 Ecological characteristics of *Cirsium* species

Species	Floral morphology	Breeding system	Pollinators	Seed dispersal	Form of clonal reproduction	Habitat
C. acaule	Gynodioecious	Predominantly outcrossing	All are insect pollinated, predominantly by bees	All are wind dispersed via a pappus	All produce new shoots from underground root and stem tissue	Closely grazed calcareous pastures
C. arvense	Incompletely dioecious	Outcrossing				Wide variety of disturbed and ruderal habitats
C. heterophyllum	Possibly hermaphrodite	No information available				Upland meadows, grasslands, streamsides, waysides and open woodland

Sources: Pigott (1968), Moore (1975), Clapham *et al*. (1981, Grime *et al*. (1989) and Jump (2002).

et al., 1999), the patterns revealed by microsatellites within species should be broadly comparable. 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 & Hamrick, 1984; Hamrick & Godt, 1996). Breeding system and floral morphology represent the principal differences between the *Cirsium* species investigated here (Table 5) and are likely to be a major contributor to the differences in structuring of genetic diversity between them (Jump, 2002).

Deviation from HWE

Unlike *C. acaule*, populations of *C. arvense* and *C. heterophyllum* do not conform to HWE. 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 (Uthicke *et al*., 1998, 1999, 2001; Ivey & Richards, 2001; Vasseur, 2001) and, consequently, F_{IS} may not be a reliable indicator of breeding system (inbreeding vs outbreeding) in such species. Bias towards heterozygote excess at both the ramet and genet level of analysis may be explained by heterozygote advantage (Lesica & Allendorf, 1992, 1995; Oostermeijer *et al*., 1994) combined with clonal selection (a gradual loss of genotypes owing to attrition, so only those genotypes that produce vigorous clonal growth remain; Schaal & Leverich, 1996). High levels of clonal reproduction in *C. arvense* and *C. heterophyllum* are also likely to explain the apparent genotypic disequilibria in these species when these do not occur in *C. acaule* (Ayer & Hughes, 2000).

Clonal diversity

There was no relationship between clonal diversity and latitude in either *C. heterophyllum* or *C. arvense*. Mean levels of clonal diversity (D, Table 2) in *C. heterophyllum* and *C. arvense* are typical of those found in species that regularly produce sexual progeny in addition to vegetative reproduction (Ellstrand & Roose, 1987). *Cirsium arvense* showed a much greater range of clonal diversity (D = 0.97–0.08) compared with *C. heterophyllum* (D = 1–0.42). *Cirsium arvense* exhibited one of the widest ranges of clonal diversity reported for any plant species (Ellstrand & Roose, 1987; Eckert & Barrett, 1993; McClintock & Waterway, 1993; Widén *et al*., 1994). By contrast to *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 multilocus genotypes in *C. arvense* has been underestimated because of the small number of loci used for genotyping individuals of this species (Eckert & Barett, 1993; McLellan *et al.*, 1997).

There is no evidence to suggest that high levels of gene flow from core populations are limiting range expansion in either *C. acaule* or *C. heterophyllum*. Although G_{ST} is only moderate (0.066) in *C. acaule*, peripheral populations of this species are highly divergent both from each other and from core populations (Fig. 3a). The high population differentiation of *C. heterophyllum* (G_{ST} = 0.359–0.318) also suggests that peripheral populations of these species are not being swamped by gene flow from core areas and hence should not lack the opportunity to adapt to range edge conditions (Hoffman & Blows, 1994; Barton, 2001).

Genetic diversity in peripheral populations

In addition to the effects of genetic drift caused by their contemporary isolation (Ellstrand & Elam, 1993; Schaal & Leverich, 1996), range-edge populations are expected to show decreased genetic diversity as a result of historic colonization processes. Genetic diversity may be lower in range edge populations both as a consequence of founder effects at expanding range margins and genetic bottlenecks at the retreating edge (Hewitt, 2000). However, only *C. acaule*, shows decreased diversity in its range-edge populations, the absence of this pattern in *C. heterophyllum* is surprising given the parallel decline in population density and seed production approaching the range edge of both species (Jump & Woodward, 2003). The loss of diversity in isolated populations may be slowed in plants that reproduce by both seed and clonal reproduction, as a consequence of clonal persistence of individuals and the increased opportunity for sexual reproduction of long-lived clones (Schaal & Leverich, 1996; Young *et al.*, 1996; Ayres & Ryan, 1997). Given the possible longevity of individual clones, very few new genets need to be added annually in order to maintain genetic diversity in such populations (Widén *et al.*, 1994; McLellan *et al.*, 1997). However, both *C. acaule* and *C. heterophyllum* demonstrate some degree of clonal reproduction, therefore this is unlikely to fully explain the disparity between these species.

Geographical structure of genetic variation

There is little agreement between the three *Cirsium* species when patterns in population structure and diversity are considered. At the largest scale, increasing geographical distance between populations is expected to result in decreasing genetic similarity (isolation by distance). This is likely to result from both historical patterns resulting from postglacial migration (Gabrielsen *et al.*, 1997; Tremblay & Schoen, 1999) and the effects of decreasing contemporary gene flow between increasingly distant populations (Schaal & Leverich, 1996). Isolation by distance is seen in both *C. acaule* and *C. arvense*, yet *C. heterophyllum* shows no such relationship (Fig. 4).

In *C. heterophyllum* there is no geographical structure when genetic distances between populations are visualized as a tree diagram (Fig. 3c). Clustering of populations is apparently random and without bootstrap support. Populations of *C. heterophyllum* from the Swiss and Italian Alps appear to show greatest genetic similarity to several Scottish populations, despite the fact that these are the most remote geographically. Tree diagrams for *C. acaule* (Fig. 3a) and *C. arvense* (Fig. 3b) also display only weak geographical structure. However, outlying populations indicated by the *C. acaule* tree diagram are those that occur at the edge of the species range, where population density of this species is lowest (Jump & Woodward, 2003). The data for *C. acaule* suggest that in accordance with predictions based on the increased isolation of populations at the range edge (Ellstrand & Elam, 1993; Schaal & Leverich, 1996), peripheral populations of *C. acaule* are divergent both from each other and from those in core areas of the species range. In *C. arvense*, the apparent outliers are also those that were sampled in the areas of its range where its frequency is lowest (north-west Scotland; Preston *et al.*, 2002), implicating population isolation in promoting population divergence in both species.

When compared with *C. acaule* and *C. arvense*, the lack of isolation by distance and the absence of geographical structure to genetic variation in *C. heterophyllum* is intriguing. Long-distance dispersal is cited by Gabrielsen *et al.* (1997) and Tollefsrud *et al.* (1998) as the cause of low geographical structure in some *Saxifraga* species, although the species investigated still demonstrate isolation by distance. The *Cirsium* species investigated here have wind-dispersible seeds, suggesting that occasional long-distance dispersal is likely (Higgins & Richardson, 1999; Cain *et al.*, 2000). Rare long-distance dispersal events may contribute to the low geographic structure of genetic variation in these species. Long-distance dispersal is unlikely to fully explain the lack of isolation by distance in *C. heterophyllum* however, as such events would need to be frequent in order to essentially randomize the geographical structure in this species; this would prevent such pronounced population differentiation (G_{ST} = 0.318–0.359). Furthermore, such events would need to cover distances as great as 1500 km (the distance between the Alps and similar central Scotland populations), such extreme dispersal distances are likely to be exceptionally rare and have not been reported for wind-dispersed plants (Cain *et al.*, 2000).

Reports of lack of isolation by distance in plant species have been attributed to factors such as rapid range expansion (*Picea abies*; Scotti *et al.*, 2000) and a combination of distributional

stasis and range fragmentation (*Anthyllis montana*; Kropf *et al.*, 2002).

Comes & Abbott (1998) cited historical long-distance dispersal and rapid range expansion as the likely cause for a lack of isolation by distance or geographical structure of allozyme variation in *Senecio gallicus*. The spatial structure of allozyme variation in *S. gallicus* in the Iberian Peninsula and southern France is almost randomized – a similar pattern to that seen in *C. heterophyllum* in the UK. Despite the lack of geographical structure in allozyme variation in *S. gallicus*, population differentiation is moderately high (F_{ST} = 0.151; Comes & Abbott, 1998). However, although little spatial structure was reported for allozyme variation in *S. gallicus* this was not the case for cpDNA or RAPD variation (Comes & Abbott, 1998, 2000), suggesting it would be advisable to determine whether greater spatial structure of genetic variation in these *Cirsium* species might be detected by alternative molecular markers.

Conclusion

Despite parallel patterns of decreasing population density and seed production approaching the edge of their geographical range, *C. acaule* and *C. heterophyllum* exhibited very different geographical patterns of genetic variation. The geographical distribution of genetic variation seen in *C. acaule* supports the expectation that peripheral populations often have low genetic diversity and are genetically divergent. The absence of such a pattern in *C. heterophyllum* suggests that this is not a general rule. Contemporary patterns of intraspecific genetic diversity clearly result from a complex interaction of historical, ecological and anthropogenic factors. Therefore, it may be misleading to make assumptions about the geographical pattern of genetic diversity within a species based solely on the present-day distribution and reproduction of its populations.

Acknowledgements

This work was supported by a Hossein Farmy scholarship from the University of Sheffield and assisted by the Sheffield Molecular Genetics Facility (supported by the Natural Environment Research Council). We thank Rajenda Whitlock for assistance in the field, Andy Krupa for help with genotyping, David Coltman for help with data analysis and Chris Eckert, Jon Slate and Lisa Pope for their comments on the manuscript.

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