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GENOTYPIC AND GENETIC DIVERSITY OF THE COMMON WEED *CIRSIUM ARVENSE* (ASTERACEAE)

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In many clonal species, seedling establishment is restricted to early successional stages when recruitment is still possible. Then, one expects that adapted genotypes become dominant and genotypic and genetic diversity should decrease with time. We investigated genotypic and genetic diversity within recently founded and established populations of the common weed *Cirsium arvense*. We used highly polymorphic amplified fragments length polymorphism (AFLP) markers. All populations were multiclonal and highly diverse (the proportion of distinguishable genotypes was 0.73 ± 0.25 [mean $\pm$ SD]). Clonal evenness was variable and ranged from 0.2 to 1. Independent of successional stage, we found on the small geographic scale of our study (<5 km) a considerable differentiation between populations ($\Phi_{SC} = 0.63$). This amount of differentiation was similar between founder and established populations and could result from selection in the early stage of succession as well as founder effects. Contrary to the general expectation, genotypic and genetic diversity were maintained through time, and molecular variance did not differ between successional stages (1.9 ± 0.89 vs. 2.5 ± 1.41). We suggest that this pattern is a consequence of the particular reproductive system of *C. arvense* that combines clonality with dioecy. The combination of clonal reproduction with the recruitment of sexually outcrossed seedlings in the first years allows the species to perform efficient colonizations even with founder effects, to undergo selection without loss of diversity, and to persist locally. This strategy appears to be very efficient in *C. arvense* and may have contributed to the worldwide success of this species.

Keywords: amplified fragments length polymorphism (AFLP), *Cirsium arvense*, clonal plant, genotypic diversity, molecular variance, population differentiation, succession.

Introduction

Clonal plants can reproduce by sexual and asexual reproduction. Whereas sexual reproduction accounts for recombination and dispersal, clonality propagates the same genotype locally. Therefore, it is often suggested that the spatial distribution of genotypic and genetic diversity within and across populations reflects the balance between clonal growth and successful sexual reproduction (Sackville Hamilton et al. 1987; Schmid 1990; McLellan et al. 1997). Alongside these two modes of reproduction, it is important to distinguish between genotypic and genetic diversity. Genotypic diversity is usually called clonal diversity in clonal plants; it stands for the number of genotypes within populations, whereas genetic diversity represents the variability between genotypes. Genetic and genotypic diversity is influenced by a number of processes (e.g., mode of reproduction, gene flow, mutation, selection). Clonal reproduction together with selection and mutation will principally affect the genotypic diversity and spatial distribution of clones within populations, whereas sexuality together with gene flow and recombination will act both on the genetic variability within and among populations.

Cirsium arvense is a long-lived perennial weed that is abundant on agricultural land. It has a mixed sexual-asexual reproduction system (Lalonde and Roitberg 1994). It reproduces vegetatively by means of very efficient lateral roots that ensure a rapid expansion of ramets. Moore (1975) reported two independent studies that found a spread by lateral roots of 6 m per season, whereas Bostock and Benton (1979) in another study noted a spread of even 12 m per year. *Cirsium arvense* is often described as a subdioecious-dioecious species. Female plants are strictly female. Male plants possess vestigial ovaries and are morphological hermaphrodites but are considered to be functional males (Moore 1975). *Cirsium arvense* is insect pollinated and produces plumed achenes with a long pappus. Natural long-distance dispersal by vegetative propagules is limited (unless roots are transported by humans, e.g., in soil); hence, new populations must be established using seeds.

In plant species with a dioecious breeding system, the genotypic diversity of seedlings establishing a new population is expected to be high compared with other less outcrossed species. However, after the establishment of seedlings, genotypes with vigorous clonal growth may be favored, as clonality is an efficient strategy to spread within a habitat. Hence,

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after some time only a few genotypes should dominate the habitat due to competitive exclusion (Gray 1987; Eriksson 1993). This process should then lead to a decrease in genotypic diversity when populations are aging, i.e., in later successional stages.

The temporal development of genotypic diversity has already been studied in other herbaceous perennials. Studies that followed genotypic diversity through time (Hartnett and Bazzaz 1985) or compared genotypic diversity between sites that differed in successional stage (McNeilly and Roose 1984; Maddox et al. 1989) showed a decline in the number of genets over time. Studies that compared populations of clonal plants in habitats with different disturbance regimes reached similar conclusions. Populations in unstable habitats tended to have a higher genotypic diversity compared with populations in stable habitats (Piquot et al. 1996; Xie et al. 2001). However, in some cases no decrease in genotypic diversity was found (Verburg et al. 2000). Some studies that followed genetic diversity through time with quantitative markers supported the theoretical predictions of a decrease in genetic diversity with time (Aarssen and Turkington 1985). In others, however, no decrease in genetic diversity over time was observed (Hartnett et al. 1987; Taylor and Aarssen 1988).

Our study is based on molecular data. We analyzed genotypic and genetic diversity within recently founded as well as established populations of *C. arvense*. Because of its high power of resolution in distinguishing genotypes, we used amplified fragment length polymorphism (AFLP) (Vos et al. 1995). Our article aims to address the following questions: (1) Is there a decrease in genotypic diversity and evenness of *C. arvense* populations with time? and (2) How is the genetic variation partitioned within and among populations?

Material and Methods

All sampled populations are located within a 15-km^2 rural area in southern Germany (11°50′E, 49°35′N). This area is a complex mosaic of roadsides, meadows, cultivated and abandoned agricultural fields, meadows, roadsides, and wasteland. In September 1999, samples were collected from 16 populations of *Cirsium arvense* from two contrasting successional stages: founder and established populations. We identified each successional stage according to information available from previous studies (Eber and Brandl 2003) and ecological criteria. Founder populations of *C. arvense* occurred in habitats where the vegetation was in a typical early successional stage. The vegetation cover was <75% and the plant community was exclusively composed of herbaceous species. *Cirsium arvense* was the dominant plant species. Shoots of *C. arvense* were easily recognizable as freshly germinated. Moreover, during our previous attempts to map all populations of *C. arvense* in the same area, these populations did not exist (Eber and Brandl 2003). Thus, we are sure that these populations date from the year of our study. Because of the difficulty in finding founder populations, we were able to sample only six populations. Established populations of *C. arvense* were sampled in old fallows. Within theses communities, *C. arvense* populations were in an advanced or regressive successional phase. There, the vegetation cover was totally closed and woody species were present. *Cirsium arvense* shoots were particularly high and ligneous. All these established populations had already been mapped at this advanced stage in 1994. Hence, populations were an age of at least 6 yr old.

In this article, we define the population size as the number of shoots within populations. In 1999, the population size of all 16 populations was estimated. For populations with <100 shoots, the number of shoots was counted and rounded off to the nearest ten. For bigger populations, the average number of plants was counted within randomly placed 1-m quadrates. Counts were extrapolated to the population area covered by *C. arvense*, with the number of shoots being rounded off to the nearest hundred or thousand.

Populations differed markedly in size, as well as in density and patchiness of shoots. Therefore, we could not apply a regular spatial sampling design. Moreover, because we were interested in estimating the genotypic diversity of *C. arvense* rather than the spatial extent of certain genotypes, we adopted a random sampling strategy. We covered each population and randomly sampled fresh leaves of *C. arvense* shoots. In order to avoid sampling the same genet twice, we limited our sampling within a patch; i.e., for an equal number of shoots, patchy populations were less sampled than uniform populations (e.g., E10 and E2). Thus, sampling effort reflects the population area and patchiness. Because of their small size ($n = 10$) we sampled almost all shoots in populations E7 and F4 (eight and seven, respectively) in order to obtain a fairly accurate value of the genotypic diversity in these populations.

The number of plants analyzed was not correlated with the population size. In order to test for a potential sampling artifact on our results, we repeated the molecular statistical analyses three times by taking only eight random samples from each population (except for populations F1 and F4, where seven samples were available). These analyses with a restricted sample size led to similar results as those from the analysis with the entire sample size (data not shown).

We extracted DNA from young leaves with CTAB (Doyle and Doyle 1988). DNA quality and concentration were estimated from 5.5 μL of the extract on an 0.8% agarose gel. For AFLP (Vos et al. 1995) we used 0.5 μg of DNA per sample. We followed the Ligation and Preselective Amplification Modules for Small Plant Genomes procedure from Applied Biosystems except that the digestion and the ligation were performed in a MWG-Biotech Primus 96 thermocycler at 37°C for 2 h. An initial screening using 64 selective primer combinations was performed on a random sample of 10 individuals across all sampled populations. From that analysis the two primer combinations EcoRI-ACC/MseI-CTG and EcoRI-ACG/MseI-CTT appeared to be sufficiently polymorphic to discriminate clones within populations. Fragments were separated on an ABI PRISM 310 genetic analyzer with 100 units as a minimum height threshold for peak detection. Data were imported to the Genotyper analysis software, but we were not able to genotype our data automatically because of the strong sensitivity of the Genotyper program and the heterogeneity among runs. Such lane-to-lane variation has already been observed in previous studies (De Riek et al. 1999). Following De Riek et al., we only used the Genotyper

program to produce a preliminary presence/absence matrix, which was subsequently checked manually. For the present study we used 93 polymorphic loci, 42 for EcoRI-ACC/MseI-CTG and 51 for EcoRI-ACG/MseI-CTT. Samples showing the same multilocus AFLP phenotype were considered to be the same genotype.

The AFLP data were analyzed at two hierarchical levels: within and among populations. As an estimator of the intrapopulation genotypic diversity we used the proportion of distinguishable genotypes ($i = G/n$; Ellstrand and Roose 1987), where G is the number of genotypes and n the number of sampled shoots. Clonal evenness was calculated as the relative abundance of each genotype within a population. A number of evenness indices are available, and there is no consensus on which one is the best (Smith and Wilson 1996). We chose the evenness index $E_{1/D} = \frac{1/D}{G}$ (Williams 1964), which is based on Simpson's index ($D = \Sigma_{i=1}^{G} p_i^2$, where p_i is the relative abundance of the ith genotype); $E_{1/D}$ ranges from 0 to 1. High values characterize populations with an even distribution of clones; low values characterize populations dominated by only a few genotypes. We compared the genotypic diversity and the evenness index between the two successional stages with a Mann-Whitney U-test.

For each population we calculated the molecular variance as the average number of mismatches of bands within a population (sum of mismatches divided by $2N\ (N-1)$, where N equals the number of samples), and we tested for correlations between the molecular variance and population size. We tested for correlations between the population size and the three factors molecular variance, genotypic diversity, and clonal evenness, using Spearman's rank correlations.

To study the partitioning of genetic variance among populations, we conducted an analysis of molecular variance (AMOVA; Excoffier et al. 1992) with the program Arlequin (Schneider et al. 2000). We constructed a hierarchical model with genotypes nested within populations and populations nested within successional stages (table 1). Variance components (σ_a^2, σ_b^2, σ_c^2; see table 2 for explanations), the sum of all squared differences, and analogues of F-statistics (Φ) were calculated. The significance of estimated parameters was tested by a permutation procedure (Excoffier et al. 1992). The parameters Φ_{CT} and σ_a^2 were tested by random permutation of genotypes of whole populations across successional stages. In this study Φ_{CT} estimated the successional stage effect. The parameters Φ_{SC} and σ_b^2 were tested by random permutation of individuals across populations but within the same successional stage. In this study Φ_{SC} estimated the population differentiation and is the equivalent of the Wright's F_{ST} index (Wright 1965). Finally, Φ_{ST} and σ_c^2 were tested by randomly permuting AFLP phenotypes among populations and between successional stages. We used a matrix correlation test with 1000 permutations to test whether the matrix of genetic distances ($\Phi_{SC}/(1-\Phi_{SC})$; Rousset 1997) was correlated with the matrix of geographic distances (table 3). The genetic distance matrix was calculated by the program Arlequin as a pairwise population Φ_{SC} matrix (Schneider et al. 2000).

Results

From the AFLPs we identified 93 polymorphic loci. Across the 307 sampled *Cirsium arvense* shoots, we distinguished 231 haplotypes (clones); 86% of these clones were found only once. Genotypes shared by several individuals (14%) always occurred in the same population. The mean genotypic diversity over all populations was $i = 0.73 \pm 0.25$ (mean $\pm$ SD). However, genotypic diversity differed considerably between populations (range: 0.25–1.0; table 1). The clonal evenness ($E_{1/D}$) was also very variable and ranged from 0.2 to 1 (mean $E_{1/D} = 0.6 \pm 0.32$). Both indices (i and E) were highly correlated (Spearman's rank correlation coefficient $r_s = 0.98$, $P < 0.0001$). Genotypic diversity and the clonal evenness did not differ significantly between founder and established populations (genotypic diversity: $U = 24$, $P > 0.3$; evenness: $U = 26.5$, $P > 0.3$) and were not correlated with the population size (genotypic diversity: $r = 0.1$; $P > 0.70$; evenness $r = 0.03$; $P > 0.92$).

Table 1

Demographic, Genotypic, and Genetic Characteristics of 16 German Populations of the Perennial Dioecious *Cirsium arvense*

Population name	Successional stage	Population size	Area (m^2)	No. of plants analyzed (n)	No. of genotypes detected (G)	Genotypic diversity (i)	Evenness index ($E_{1/D}$)	Molecular variance
F1	Founder	30	6	7	6	0.86	0.78	2.12
F2	Founder	40	25	15	7	0.47	0.28	0.48
F3	Founder	200	40	17	16	0.94	0.89	4.59
F4	Founder	10	10	7	7	1.00	1	3.45
F5	Founder	100	80	19	9	0.47	0.2	1.78
F6	Founder	120	50	10	10	1.00	1	2.63
E1	Established	500	750	53	42	0.79	0.58	2.48
E2	Established	100	160	20	12	0.60	0.29	1.93
E3	Established	30	200	20	20	1.00	1	3.01
E4	Established	3000	1200	54	52	0.96	0.93	2.92
E5	Established	400	300	28	13	0.46	0.29	1.07
E6	Established	40	50	12	6	0.50	0.33	1.06
E7	Established	10	10	8	2	0.25	0.2	0.75
E8	Established	800	130	11	11	1.00	1	2.42
E9	Established	8000	1000	18	13	0.72	0.5	0.76
E10	Established	100	80	8	5	0.63	0.5	2.39

Table 2

Hierarchical Analysis of Molecular Variance Testing for Differentiation between Successional Stages, among Populations within Successional Stages, and within Populations

Variation component		Variance	% Total	Significance	Φ-Statistics
Between successional stages	σ_a^2	0.58	5	$P < 0.01$	$\Phi_{CT} = 0.05$
Among populations within successional stages	σ_b^2	7.46	60	$P < 0.001$	$\Phi_{SC} = 0.63$
Within populations	σ_c^2	4.44	35	$P < 0.001$	$\Phi_{ST} = 0.64$

Note. Significance tests are based on 1000 permutations.

The average molecular variance was 2.1 (±1.1). Established populations had a lower mean molecular variance than the founder populations (1.9 ± 0.89 vs. 2.5 ± 1.41), but the difference was not significant ($U = 23$, $P > 0.44$). Molecular variance was not correlated with the population size (fig. 1). Most of the haplotype diversity was found among populations within successional stages ($\sigma_b^2 = 60\%$), which results in a high Φ_{SC} value (0.63). Nevertheless, a considerable amount of diversity was found within populations ($\sigma_c^2 = 35\%$). The variance from differentiation between founder and established populations was significant but explained only a small part of the total variance ($\sigma_a^2 = 5\%$).

Pairwise genotypic distances were not correlated to geographic distances (matrix correlation = 0.14, $P > 0.13$; fig. 2).

Discussion

Genotypic diversity and clonal evenness of *Cirsium arvense* were high and varied greatly among the 16 sampled populations. These results agree with recent data based on microsatellites. Jump et al. (2003) found an average genotypic diversity of 0.68 (ranging from 0.08 to 0.97) and an average evenness of 0.68 (ranging from 0 to 1). Direct comparisons with other data from the literature are difficult to interpret because reviews are based on different sample sizes, spatial scales, and molecular markers and compare plant species with different life and phylogenetic histories (Gitzendanner and Soltis 2000). However, compared to the most widely cited reviews of genetic diversity patterns in clonal plants (Ellstrand and Roose 1987; Widen et al. 1994), average genotypic diversity in *C. arvense* is much higher (0.73 vs. 0.17). This big difference found with AFLP and microsatellites might result from the higher power of resolution of the DNA-based molecular markers, whereas the abovementioned reviews are mostly based on allozyme studies, which offer fewer loci. Nevertheless, the high genotypic diversity found in *C. arvense* is confirmed by the fact that 100% of the sampled populations were multiclonal, compared to an average of 62% in Ellstrand and Roose (1987) and Widen et al. (1994).

Like genotypic diversity, the clonal evenness may vary greatly among clonal species. Populations can be mono- or multiclonal (Aspinwall and Christian 1992; Eckert and Barrett 1993; McClintock and Waterway 1993; Piquot et al. 1996). For multiclonal populations, almost all patterns of genotypic diversity combined with clonal evenness can be found (Ayres and Ryan 1997; Gabrielsen and Brochmann

Table 3

Matrices of Pairwise Genetic and Geographic Distances among 16 German Populations of *Cirsium arvense*

	F1	F2	F3	F4	F5	F6	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10
F1		0.13	1.83	0.73	0.85	0.88	0.70	0.72	0.60	0.59	0.82	0.80	0.83	2.95	0.80	0.69
F2	0.87		1.80	0.75	0.75	0.83	0.62	0.76	0.70	0.58	0.85	0.87	0.94	3.05	0.88	0.82
F3	0.34	0.55		2.28	2.48	2.03	0.53	0.46	0.44	0.43	0.58	0.53	0.65	1.68	0.57	0.43
F4	0.53	0.77	0.22		1.25	1.53	0.56	0.52	0.50	0.47	0.74	0.66	0.79	3.00	0.73	0.43
F5	0.71	0.80	0.44	0.43		0.63	0.63	0.56	0.64	0.60	0.78	0.77	0.85	3.75	0.77	0.68
F6	0.61	0.73	0.35	0.51	0.64		0.55	0.66	0.57	0.50	0.77	0.74	0.81	3.48	0.77	0.62
E1	3.13	3.15	2.58	3.85	3.08	2.48		0.56	0.62	0.49	0.64	0.60	0.77	4.13	0.65	0.60
E2	3.15	3.25	1.55	3.35	3.90	3.50	3.80		2.15	1.80	0.66	0.72	0.82	0.65	0.75	0.60
E3	1.25	1.35	0.63	1.78	1.85	1.40	2.48	0.58		0.38	0.73	0.65	0.75	2.15	0.67	0.49
E4	1.58	1.68	0.28	2.05	2.20	1.75	2.50	0.51	0.50		0.63	0.60	0.72	1.88	0.64	0.54
E5	2.80	2.98	1.55	3.55	3.25	2.65	1.50	2.35	1.80	1.60		0.28	2.18	2.83	2.20	1.70
E6	2.65	2.73	1.35	3.33	3.00	2.38	1.45	2.35	1.53	1.38	0.78		2.13	2.75	1.75	1.50
E7	2.60	2.73	1.10	2.83	3.38	3.00	3.53	0.53	1.63	1.65	0.90	0.89		0.63	3.43	3.20
E8	0.63	0.77	0.45	0.53	0.71	0.60	0.61	0.62	0.57	0.56	0.70	0.71	0.82		0.72	0.55
E9	2.28	2.25	2.35	3.00	2.08	1.50	1.05	3.80	1.98	2.15	0.82	0.83	0.91	4.00		0.25
E10	2.23	2.25	2.13	2.95	2.13	1.53	0.95	3.55	1.80	1.95	0.79	0.71	0.79	3.75	0.79	

Note. Genetic distances (mean number of pairwise mismatches) are presented in the lower part of the table; geographic distances (km) are in the upper part.

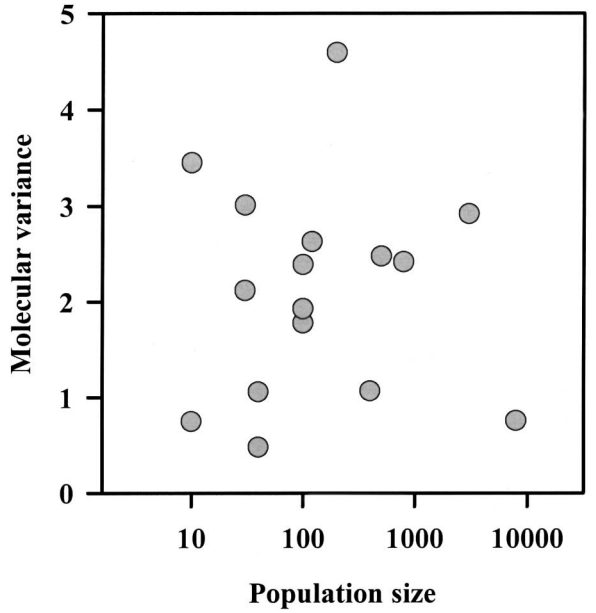

Fig. 1 Population size (number of shoots counted per population) in 16 German populations of *Cirsium arvense* was not correlated with the molecular variance ($r_s = 0.09$, $P > 0.72$).

1998; Ivey and Richards 2001*b*). In *C. arvense*, the genotypic diversity is high, and populations are not dominated by one clone, a pattern that also was found in other species (Chung and Epperson 1999; Auge et al. 2001; Xie et al. 2001).

Population differentiation frequently has been analyzed with allozymes. Three main results emerged: differentiation between populations is rather similar in plant species with mixed and purely sexual reproduction (G_{ST} values 0.21 and 0.23, respectively; Hamrick and Godt 1990); most variation occurs within populations (Baur and Schmid 1996); and population differentiation is lower in outbreeding than in inbreeding species (G_{ST} values <20% and >50%, respectively). Studies based on dominant markers (RAPD) lead to the same conclusions (Bussel 1999). In *C. arvense* most of the genetic variation was among populations (60% of the total variation). All haplotypes were strictly local. The Φ_{SC}-statistic (equivalent in our study to Wrights's F_{ST} value) was very high ($\Phi_{SC} = 0.64$), even compared with previous studies (0.25 in Jump et al. 2003).

At a landscape scale, we found that differentiation among populations was not correlated to geographic distance. These results fortify those of Jump et al. (2003), who observed an isolation by distance in *C. arvense* occurring from 200 km. A high differentiation among populations independent of geographic distance, like in *C. arvense*, is more common in rare, partially selfing, locally dispersed, or gene flow limited species (Travis et al. 1996; Fischer and Matthies 1998; Schmidt and Jensen 2000). Thus, the high population differentiation independent of geographic distance in *C. arvense* is surprising because *C. arvense* is an abundant outbreeding species.

In plants with mixed reproduction systems, clonal propagation can bias the estimation of *F*-statistics (McLellan et al. 1997). For example, in the clonal *Cladium jamaicense* the F_{ST} based on the ramet (i.e., all aerial shoots coming from the clonal propagation of a single root) was 0.68, whereas the F_{ST} based on the genets (genetic individuals) was 0.035 (Ivey and Richards 2001*a*). In *C. arvense*, the analysis on the genet level also resulted in a high population differentiation ($\Phi_{SC} = 0.53$, $P < 0.001$). Therefore, clonality is not the reason for population differentiation, a result already evident from the fact that populations were not dominated by one or a few clones. In addition to clonality, high population differentiation independent of geographic distance can also result from strong selection (Endler 1986) as well as from founder effects acting together with drift, low gene flow among populations, or low seedling recruitment within populations (Slatkin 1977; Whitlock 1992).

Similarity among genotypes within populations, and thus dissimilarity among populations, is expected to increase in small and isolated populations as a consequence of genetic drift, bottlenecks, and inbreeding (Hartl and Clark 1989; Barrett and Kohn 1991). A decline in molecular variance in small populations was frequently found, e.g., in the nonclonal *Gentianella germanica* (Fischer and Matthies 1997) as well as in the clonal herb *Ranunculus reptans* (Fischer et al. 2000). In our study, population size was correlated neither with molecular variance nor with genotypic diversity. The correlation performed on established populations only, which are more likely to have experienced genetic drift, was also not significant. Thus, genetic drift does not seem to play an important role in population differentiation.

Gene flow among populations can be distinguished into pollen and seeds. The pollinators of *C. arvense*, mainly bumble bees, are very mobile (Walther-Hellwig and Frankl 2000). The observation that seeds were produced in all populations

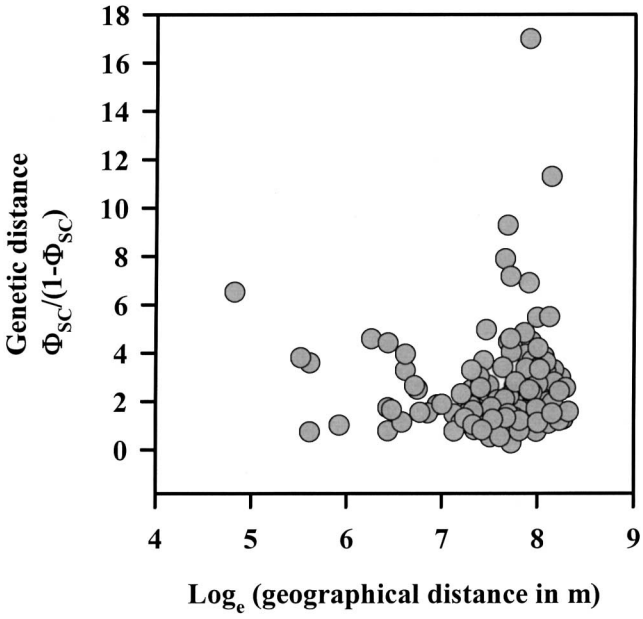

Fig. 2 Genetic and geographic distances in 16 German populations of *Cirsium arvense* were not correlated (matrix correlation = 0.12, $P > 0.18$). High population differentiation observed in *C. arvense* is not due to geographic distance.

even with 100% of females (M. Solé, unpublished manuscript) and the fact that apomixis does not occur (Correns 1916) confirm an efficient transport of pollen among populations. However, low seed dispersal and absence of seedling recruitment in *C. arvense* are potential causes that generate and maintain differentiation. Despite producing plumed seeds, seed dispersal in *C. arvense* may not be very effective. The pappus often breaks off the seed, so that at a distance of 1000 m only 0.2% of trapped pappi carried a seed (Bakker 1960; Bostock and Benton 1979). Like in other clonal plants (Eriksson 1992; Wolf et al. 2000), the recruitment of *C. arvense* seedlings is possible only during the early phases of colonization. *Cirsium arvense* seedlings are very susceptible to shading and competition (Bakker 1960). Our observations as well as reports in the literature indicate that the recruitment of *C. arvense* seedlings is not possible in natural or artificial plant communities with a dense plant cover (Bostock and Benton 1983; M. Solé, unpublished data). Thus, once populations have reached a closed canopy, seedling recruitment will stop. This phenomenon could then reinforce the population differentiation.

Two potential driving forces remain to explain the surprisingly high genetic differentiation among *C. arvense* populations: founder effects and selection. We tested for founder effects (i.e., restricted-source origin of founder populations) by comparing the level of genetic differentiation among founder and established populations (Whitlock and McCauley 1990). The Φ_{SC} values were 0.55 ($P < 0.001$) for founder populations versus 0.64 ($P < 0.001$) for established populations. As differentiation among founder populations was already high, this result may indicate the foundation of new populations by a nonrandom sample of the propagule pool of *C. arvense*.

In *C. arvense*, founder effects and selection are not mutually exclusive. However, founder effects and strong selection usually tend to decrease the within-population genetic variability (Endler 1986; Pannell and Charlesworth 1999). In our study, genotypic and genetic diversity did not decrease from founder to established populations. We offer the following explanation. We sampled plants in September, and thus the founder populations already passed the seedling stage. If mortality and strong selection occur during the seedling stage, our sampling scheme was not able to retrieve the decrease of genetic diversity because the founder populations had already passed the filter of a first selection phase. As long as the selection regime is not spatially autocorrelated, this will lead to high differentiation among populations.

However, the maintenance of genetic diversity through time could also be fostered by the obligatory outcrossed breeding system of *C. arvense*. Comparable results (high genetic diversity coupled with a high differentiation of populations) were already found in other clonal dioecious species (Sherman-Broyles et al. 1992 in *Rhus* species; Gibson and Wheelwright 1995). Because of dioecy, *C. arvense* seeds are strictly outcrossed and thus must be highly variable. Their recruitment during the early stages of succession could compensate for the loss of diversity within a population. Therefore, seedling recruitment in *C. arvense* might be an important mechanism for initiating and maintaining a high level of neutral genetic variability, as already suggested by Heimann and Cussans (1996). Recently, theoretical studies about the genetic diversity in clonal plants led to similar conclusions (Bengtsson 2003). In his model, Bengtsson looked at the "genotypic identity" of populations (i.e., the probability that two randomly sampled adult individuals from a population have the same genotype) depending on the population growth parameters, rate of sexuality, and recruitment of sexually derived offspring. From his simulations, Bengtsson proposed that clonal populations possess an effective "memory" of their earlier genetic history, in the way that "a population which was started by a number of sexually derived propagules may thus retain its initial genotypic variation for a very long period of time, even if it later reproduces almost exclusively asexually" (p. 196).

Conclusions

In contrast to the expected decline of genotypic diversity over time, genotypic diversity, and evenness did not vary between founder and established populations of *Cirsium arvense*. On a small geographic scale, sampling at the same time, we found an extremely high population differentiation together with a high within-population variability. We interpret these patterns of genotypic and genetic diversity as the result of the particular reproductive system of the species. Founder effects and early selection in the seedling stage may contribute to genetic differentiation among populations. Then a combination of recruitment of sexually outcrossed seedlings in the early stages of succession and clonal reproduction allows the species to perform efficient colonization and to persist locally. The diversity of genotypes as well as genetic diversity are maintained through time and mainly seem to reflect the status built up during the early stage of succession. This strategy appears to be very efficient in *C. arvense*, and it may have contributed to the worldwide success of this species.

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