# Chapter I

## Genotypic and genetic diversity of the common weed *Cirsium arvense* (Asteraceae)

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**Abstract:** 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 AFLP markers. All populations were multiclonal and highly diverse (proportion of distinguishable genotypes was 0.73 ± 0.25 (mean ± 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 selection, 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 versus 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 recruitment of sexually outcrossed seedlings in the first years allows the species to perform efficient colonisation 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.

**Key words:** 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 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). In correlation with 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 and stands for the number of genotypes within populations, whereas genetic diversity represents the variability between genotypes. 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 more on the genetic variability within and among populations.

*C. arvense* is a long-lived perennial weed, abundant in agricultural land. It has a wide distribution in Europe from the Atlantic ocean to the Ural mountains. *C. arvense* is also widely distributed in North America where it has been introduced in the 17$^{th}$ century. Since the introduction, *C. arvense* has spread throughout Canada and the United States where it is one of the most tenacious and economically important weeds (Donald, 1990). *C. arvense* has a mixed sexual-asexual reproduction system. It reproduces vegetatively by means of very efficient lateral roots which assure a rapid expansion of ramets. Moore (1975) reported on two independent studies which found a spread via lateral roots of 6 m per season, whereas in Bostock and Benton (1979) another study even note a spread of 12 m a year. *C. 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). *C. arvense* is insect-pollinated and produces plumed achenes with a long pappus. Natural long distance dispersal by vegetative propagules is limited (except if roots are transport by man e.g. in soil), hence new populations must be founded by seeds.

Because of its dioecious breeding system, genotypic diversity of seedling founding a new population is expected to be high compared to other none strictly out-crossed species. However, after the establishment of seedlings, genotypes with vigorous clonal growth may become favoured, as clonality is a better strategy to spread and colonize a habitat for plants

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

Studies that followed genotypic diversity through time (e.g. Hartnett and Bazzaz, 1985 in *Solidago canadensis*) or compared genotypic diversity between sites which differed in successional stage (e.g. Maddox et al., 1989 in *Solidago altissima*; McNeilly and Roose, 1984 in *Lolium perene*) showed a decline in the number of genets over time. Studies, which compared populations of clonal plants in habitats with different disturbance regime reached similar conclusions. Populations in unstable habitats tended to have a higher genotypic diversity compared to populations in stable habitats (Piquot et al., 1996, Xie et al., 2001). However, in some cases no decrease of genotypic diversity was found (Verburg et al., 2000). Some studies which followed genetic diversity through time with quantitative markers supported the theoretical predictions (Aarssen and Turkington, 1985). But in some others no decrease of genetic diversity over time was observed (Taylor and Aarssen, 1988; Hartnett et al., 1987).

The present study is based on molecular data. We looked at genotypic and genetic diversity within recently founded as well as established populations of *C. arvense*. Because of their high power of resolution in distinguishing genotypes, we used Amplified Fragment Length Polymorphism (Vos et al. 1995). Our paper aims to address the following questions: (1) Is there a decrease in genotypic diversity and evenness of *C. arvense* populations with time? (2) How is the genetic variation partitioned within and among populations?

## Material and methods

### *Sampling*

All sampling populations belong to a 15 km² rural area located in Southern Germany (11°50'E 49°35'N). This area is a complex mosaic of roadsides, meadows, cultivated and abandoned agricultural fields, wastelands. In September 1999, samples were collected from 16 natural populations of *C. arvense* from two contrasting successional stages: founder and established. We identified each successional stage according to information available from previous studies (Eber and Brandl, 1994; Eber and Brandl, 1996) 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 below 75 % and the plant community was exclusively composed of herbaceous species. *C. arvense* was the dominant plant species. Shoots of *C. arvense* were easily recognizable as freshly germinated as they significantly differ from shoots from mature successional stages (Solé in prep). Moreover, during our previous attempts to map all populations of *C .arvense* in the same area these populations were not existent (Eber and Brandl, 2003). Thus, we are sure that these populations date from the year of our study. Because of the difficulty to find 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. *C. arvense* shoots were particularly high and ligneous. All these established populations were already mapped in this advanced stage in 1994. Although the date of foundation of these populations is unknown, we can guess they have been found for 10 years.

In 1999, the size (number of shoots) of all 16 populations was recorded. For populations having less than 100 shoots, the number of shoots was counted and rounded to the nearest ten. For bigger populations, average number of plants was counted in one square meter and extrapolated to the population area covered by *C. arvense*. In these cases, the number of shoots was rounded to the nearest hundreds or thousands.

Populations differed markedly in their size, density and patchiness. Therefore, we could not apply a regular spatial sampling design to all populations. Moreover, as 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 randomly sampled fresh leaves of

*C. arvense* shoots according to population extent and patchiness in order to have a sampling effort reflecting the ramet density (Table 1).

**Table 1**. Demographic, genotypic and genetic characteristics of 16 German populations of the clonal dioecious *Cirsium arvense.*

| Population name | Successional stage | Population size (number of shoots) | Number of plants analysed (N) | Number of genotypes detected (G) | Genotypic diversity (i) | Evenness index ($E_{1/D}$) | Molecular variance |
|---|---|---|---|---|---|---|---|
| F1 | Founder | 30 | 7 | 6 | 0.86 | 0.78 | 2.12 |
| F2 | Founder | 40 | 15 | 7 | 0.47 | 0.28 | 0.48 |
| F3 | Founder | 200 | 17 | 16 | 0.94 | 0.89 | 4.59 |
| F4 | Founder | 10 | 7 | 7 | 1.00 | 1 | 3.45 |
| F5 | Founder | 100 | 19 | 9 | 0.47 | 0.2 | 1.78 |
| F6 | Founder | 120 | 10 | 10 | 1.00 | 1 | 2.63 |
| E1 | Established | 500 | 53 | 42 | 0.79 | 0.58 | 2.48 |
| E2 | Established | 100 | 20 | 12 | 0.60 | 0.29 | 1.93 |
| E3 | Established | 30 | 20 | 20 | 1.00 | 1 | 3.01 |
| E4 | Established | 3000 | 54 | 52 | 0.96 | 0.93 | 2.92 |
| E5 | Established | 400 | 28 | 13 | 0.46 | 0.29 | 1.07 |
| E6 | Established | 40 | 12 | 6 | 0.50 | 0.33 | 1.06 |
| E7 | Established | 10 | 8 | 2 | 0.25 | 0.2 | 0.75 |
| E8 | Established | 800 | 11 | 11 | 1.00 | 1 | 2.42 |
| E9 | Established | 8000 | 18 | 13 | 0.72 | 0.5 | 0.76 |
| E10 | Established | 100 | 8 | 5 | 0.63 | 0.5 | 2.39 |

***DNA extraction and AFLP***

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 a 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 during 2 hours. 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 analyser with 100 units as a minimum height threshold for peak detection. Data were imported to the

analysing software *Genotyper*, 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 a lane-to-lane variation has been already 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 EcoRI-ACG / MseI-CTT. Samples showing the same multilocus AFLP phenotype were considered to be the same genotype.

***Analyses***

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 choose the evenness index $E_{1/D} = \frac{1/D}{G}$ (Williams, 1964), which is based on Simpson's index ($D = \sum_{i=1}^{G} p_i^2$ where $p_i$ is the relative abundance of the $i^{th}$ 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 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 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 stage (Table 1). Variance components ($\sigma_a^2$, $\sigma_b^2$, $\sigma_c^2$; see Table 3 for explanations), the sum of all squared differences and analogs of F-

statistics (Φ) were calculated. The significance of estimated parameters was tested by a permutation procedure (Excoffier et al., 1992). $\Phi_{ct}$ and $\sigma_a^2$ were tested by random permutation of genotypes of whole populations across successional stages. In this study $\Phi_{ct}$ estimated the successional stage effect. $\Phi_{sc}$ and $\sigma_b^2$ were tested by random permutation of individuals across populations but within the same successional stage. $\Phi_{sc}$ estimated the population differentiation and is the equivalent of the Wright's $F_{st}$ index (Wright, 1965). $\Phi_{st}$ and $\sigma_c^2$ were tested by permuting randomly 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 2). The genetic distance matrix was calculated by the program Arlequin as a pairwise population $\Phi_{sc}$ matrix (Schneider et al., 2000).

**Table 2**: Matrices of pairwise genetic and geographic distances among 16 German populations of *Cirsium arvense*. Genetic distances ($\Phi_{sc}$) are presented in the lower part of the table; the geographic distances (km) in the upper part.

| | F1 | F2 | F3 | F4 | F5 | F6 | E1 | E2 | E3 | E4 | E5 | E6 | E7 | E8 | E9 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| F1 | | 0.13 | 1.83 | 0.73 | 0.85 | 0.88 | 0.7 | 0.72 | 0.6 | 0.59 | 0.82 | 0.8 | 0.83 | 2.95 | 0.8 |
| F2 | 0.87 | | 1.8 | 0.75 | 0.75 | 0.83 | 0.62 | 0.76 | 0.7 | 0.58 | 0.85 | 0.87 | 0.94 | 3.05 | 0.88 |
| 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 |
| F4 | 0.53 | 0.77 | 0.22 | | 1.25 | 1.53 | 0.56 | 0.52 | 0.5 | 0.47 | 0.74 | 0.66 | 0.79 | 3 | 0.73 |
| F5 | 0.71 | 0.80 | 0.44 | 0.43 | | 0.63 | 0.63 | 0.56 | 0.64 | 0.6 | 0.78 | 0.77 | 0.85 | 3.75 | 0.77 |
| F6 | 0.61 | 0.73 | 0.35 | 0.51 | 0.64 | | 0.55 | 0.66 | 0.57 | 0.5 | 0.77 | 0.74 | 0.81 | 3.48 | 0.77 |
| E1 | 0.70 | 0.62 | 0.53 | 0.56 | 0.63 | 0.55 | | 0.56 | 0.62 | 0.49 | 0.64 | 0.6 | 0.77 | 4.13 | 0.65 |
| E2 | 0.72 | 0.76 | 0.46 | 0.52 | 0.56 | 0.66 | 0.56 | | 2.15 | 1.8 | 0.66 | 0.72 | 0.82 | 0.65 | 0.75 |
| E3 | 0.60 | 0.70 | 0.44 | 0.50 | 0.64 | 0.57 | 0.62 | 0.58 | | 0.38 | 0.73 | 0.65 | 0.75 | 2.15 | 0.67 |
| E4 | 0.59 | 0.58 | 0.43 | 0.47 | 0.60 | 0.50 | 0.49 | 0.51 | 0.50 | | 0.63 | 0.6 | 0.72 | 1.88 | 0.64 |
| E5 | 0.82 | 0.85 | 0.58 | 0.74 | 0.78 | 0.77 | 0.64 | 0.66 | 0.73 | 0.63 | | 0.28 | 2.18 | 2.83 | 2.2 |
| E6 | 0.80 | 0.87 | 0.53 | 0.66 | 0.77 | 0.74 | 0.60 | 0.72 | 0.65 | 0.60 | 0.78 | | 2.13 | 2.75 | 1.75 |
| E7 | 0.83 | 0.94 | 0.65 | 0.79 | 0.85 | 0.81 | 0.77 | 0.82 | 0.75 | 0.72 | 0.90 | 0.89 | | 0.63 | 3.43 |
| 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 |
| E9 | 0.80 | 0.88 | 0.57 | 0.73 | 0.77 | 0.77 | 0.65 | 0.75 | 0.67 | 0.64 | 0.82 | 0.83 | 0.92 | 0.72 | |
| E10 | 0.69 | 0.82 | 0.43 | 0.43 | 0.68 | 0.62 | 0.60 | 0.60 | 0.49 | 0.54 | 0.79 | 0.71 | 0.79 | 0.55 | 0.79 |

## Results

From our AFLPs we identified 93 polymorphic loci. We distinguished 231 haplotypes (clones) across the 307 sampled *C. arvense* shoots; 86 % of these clones were found only once. Genotypes shared by several individuals (14%) always occurred in the same population. The number of clones per population ranged from 2 to 52. The mean genotypic diversity over all populations was i = 0.73 ± 0.25 (mean ± 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 ± 0.32). Both indices (i and E) were highly correlated (Spearman 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; $P > 0.92$).

The average molecular variance was 2.1 (± 1.1). Established populations had a lower mean molecular variance than the founder ones (1.9 ± 0.89 versus 2.5 ± 1.41) but the difference was not significant (U = 23; $P > 0.44$). Molecular variance was not correlated with the population size (Figure 1).

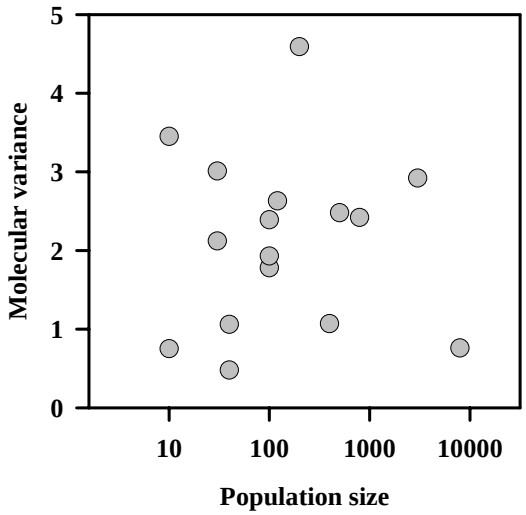


**Figure 1**: Population size (number of shoots counted per population) in 16 German populations of *Cirsium arvense* was not correlated with the molecular variance ($r_s$ = 0.09; $P > 0.72$).

Most of the haplotype diversity was found among populations within successional stages ($\sigma_b^2$ = 60 %; Table 3), which results in a high $\Phi_{sc}$ value (0.63). Nevertheless, a considerable amount of diversity was found within populations ($\sigma_c^2$ = 35 %; Table 3). The variance due to differentiation between founder and established populations was significant but explained only a small part of the total variance ($\sigma_a^2$ = 5 %; Table 3).

**Table 3**. Hierarchical analysis of molecular variance testing for differentiation between successional stages, among populations within successional stages and within populations. The significance tests are based on 1000 permutations.

| Variation Component | | Variance | % total | Significance | Φ-statistics |
|---|---|---|---|---|---|
| Between successional stages | $\sigma_a^2$ | 0.58 | 5 | P < 0.01 | $\Phi_{ct}$ = 0.05 |
| Among populations within successional stages | $\sigma_b^2$ | 7.46 | 60 | P < 0.001 | $\Phi_{sc}$ = 0.63 |
| Within populations | $\sigma_c^2$ | 4.44 | 35 | P < 0.001 | $\Phi_{st}$ = 0.64 |

Pairwise genotypic distances were not correlated to geographic distances (matrix correlation = 0.25, *P* > 0.2; Figure 2).

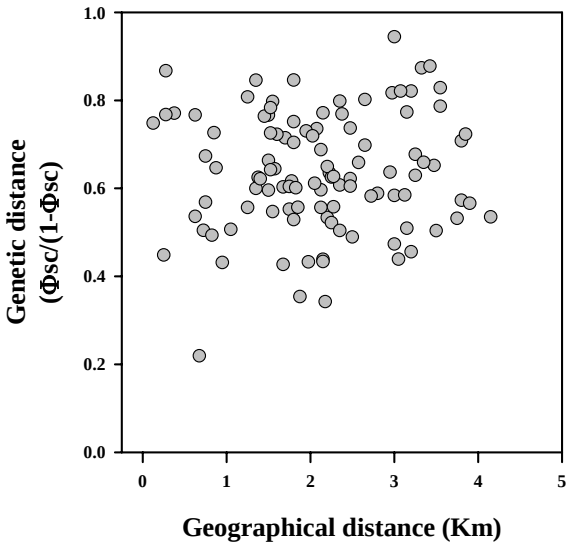


**Figure 2**: The relation between the genetic and the geographical distances in 16 German populations of *Cirsium arvense* show no correlation between the two matrices (matrix correlation = 0.12; *P* > 0.18). High population differentiation observed in *C. arvense* is not due to geographical distances.

## Discussion

Genotypic diversity and clonal evenness of *C. arvense* varied greatly among the sixteen sampled populations. The average genotypic diversity was equal to 0.73. Direct comparisons with data from the literature is difficult to interpret as reviews are based on different sample sizes, spatial scales, 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 and Widen et al., 1994) average genotypic diversity in *C. arvense* is much higher (0.73 against 0.17). This big difference might mostly result from the highest power of resolution of the DNA-based molecular markers (we had 93 polymorphic loci), whereas the above-mentioned reviews are mostly based on allozymes 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, against 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 (Piquot et al., 1996; Eckert and Barrett, 1993; Aspinwall and Christian, 1992; McClintock and Waterway, 1993). For multiclonal populations, almost all patterns of genotypic diversity combined with clonal evenness can be found. (Ayres and Ryan, 1997, Ivey and Richards, 2001b; Gabrielsen and Brochmann, 1998). In *C. arvense*, the genotypic diversity is high and populations are not dominated by one clone, a pattern which was also found in other species (Chung and Epperson, 1999; Xie et al., 2001; Auge et al., 2001).

Population differentiation has frequently been analyzed with allozymes. Three main results emerged: (1) 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) (2) most variation occurs within populations (Baur and Schmid, 1996) (3) 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 $\Phi_{sc}$-statistic (equivalent in our study to Wrights' $F_{st}$ value) was very high ($\Phi_{sc}$ = 0.64). Furthermore, differentiation among

populations was not correlated to geographic distance. A high differentiation among populations independent of geographical distance, like in *C. arvense*, is more common in rare, partially selfing, locally dispersed, or gene flow limited species (Fischer and Matthies, 1998; Travis et al., 1996; Schmidt and Jensen, 2000). Thus, the high population differentiation independent of geographical distance in *C. arvense* is surprising, as *C. arvense* is an abundant outbreeding species.

In plants with mixed reproduction systems, clonal propagation can strongly 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 Fst based on the genets (genetic individuals) was 0.035 (Ivey and Richards, 2001a). 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 of population differentiation. A result, which was already evident from the fact that populations were not dominated by one or few clones.

Besides clonality, high population differentiation independent of geographical 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 non-clonal *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 neither correlated with the molecular variance, nor with the genotypic diversity. The same correlation performed on established populations only, which are more likely to have experienced genetic drift, was not significant. Hence, *C. arvense* populations need not have been strongly affected by non-selective processes through time.

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 even with 100 % of females (Solé unpublished data) and the fact that apomixis does not occur (Solé personal observation)

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, the effectiveness of seed dispersal in *C. arvense* is arguable. 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 (Wolf et al., 2000, Eriksson, 1992) the recruitment of *C. arvense* seedlings is possible only during the early phases of the colonization. *C. 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; Solé unpublished data). Thus, once populations have reached closed canopy seedling recruitment will stop. This phenomenon could then reinforce the population differentiation (see Gibson and Wheelwright 1995).

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 $\Phi_{st}$ 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 foundation of new populations by a non random sample of the propagule pool of *C. arvense*.

Selection in *C. arvense* can also be an important factor, which designs the genetic differentiation between populations. Strong selection (e.g. for genotypes that have the highest capacity for clonal growth) can occur in the early stages of succession. During this phase, many genotypes may become established but seedling mortality is high (Brandl et al. unpublished data).

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 (Pannell and Charlesworth, 1999; Endler, 1986). 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 as 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 between 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). 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 the loss of diversity within population, and therefore maintain a high level of neutral genetic variability. Recently theoretical studies about the genetic diversity in clonal plants lead to similar conclusions (Bengtsson, 2003). In his model, Bengtsson looked at the "Genotypic Identity" of population (i.e. the probability that two randomly sampled adult individuals from a population have the same genotype) depending on 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 started by a number of sexually derived propagules may thus retain its initial genotypic variation for a very long period of time, even if its later reproduces almost exclusively asexually".

## Conclusion

In contrast to the expected decline of genotypic diversity over time, genotypic diversity and evenness did not vary between founder and established populations of *C. arvense*. We found on a small geographic scale at the same time 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, 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. 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 may have contributed to the worldwide success of this species.

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