Diversity and spatial structure of clones in *Vaccinium uliginosum* populations

T. Albert, O. Raspé, and A.-L. Jacquemart

Abstract: In Belgium, a total of three *Vaccinium* species with a shrub or dwarf shrub growth form can be found: *Vaccinium myrtillus* L., *Vaccinium vitis-idaea* L., and *Vaccinium uliginosum* L. *Vaccinium uliginosum* is the only one of these for which the extent of clonality is unknown. Therefore, the clonal structure of two *V. uliginosum* populations was inferred from random amplified polymorphic DNA (RAPD) markers. Among the 47 sampled patches, 61 clones were identified. The mean values of the number of clones divided by the number of samples (*G/N*), the Simpson's index (*D*), and the genotypic evenness (*E*) were 0.28, 0.95, and 0.96, respectively. *Vaccinium uliginosum* exhibited a typical phalanx growth strategy that resulted in structured populations at the ramet level, that is, ramets belonging to the same clone were closely associated and formed distinct clumps. However, at the clone level, populations were not structured, that is, genetic distances between pairs of clones were not correlated with the spatial distances between the clones within a population. Genetic diversity was as high as that in nonclonal species (mean value of Shannon's diversity index (H_o) = 0.647). In accord with the life history traits of *V. uliginosum* (long-lived species with a mixed breeding system and potentially high seed dispersal), most of the genetic variation was found within populations.

Key words: genetic diversity, genetic structure, seedling recruitment, vegetative propagation, Ericaceae.

Résumé : Trois espèces de *Vaccinium* à port buissonnant (buisson ou buisson nain) sont présentes en Belgique : *Vaccinium myrtillus* L., *Vaccinium vitis-idaea* L. et *Vaccinium uliginosum* L. *Vaccinium uliginosum* est la seule de ces trois espèces pour laquelle aucune étude sur la clonalité n'a été réalisée. Par conséquent, nous avons analysé la structure clonale de deux populations du *V. uliginosum,* à l'aide de marqueurs RAPD (random amplified polymorphic DNA). Parmi les 47 colonies échantillonnées, 61 clones ont été identifiés. Les valeurs moyennes du nombre de clones divisé par le nombre d'échantillons (*G/N*), de l'indice de Simpson (*D*) et de l'uniformité génotypique (*E*) sont respectivement égales à 0,28, 0,95 et 0,96. *Vaccinium uliginosum* présente une forme de croissance typiquement en phalangée, ce qui donne une structure aux populations au niveau des ramètes, les ramètes appartenant au même clone étant juxtaposées. Cependant, au niveau des clones, les populations ne sont pas structurées, les distances génétiques observées entre paires de clones, au sein d'une population, n'étant pas corrélées aux distances spatiales séparant ces clones. La diversité génétique est aussi élevée que celle observée pour des espèces non-clonales (valeur moyenne de l'indice de diversité de Shannon (H_o) = 0,647). La plupart de la variation génétique est présente au sein des populations, ce qui est cohérent avec les traits d'histoire de vie de l'espèce (espèce à longue durée de vie ayant un système de reproduction mixte et une dispersion de graines potentiellement élevée).

Mots clés : diversité génétique, structure génétique, recrutement de plantules, multiplication végétative, Ericaceae.

Introduction

Because of the hierarchical organisation of clonal plants ("genets" and "ramets"), two levels of molecular variation can be distinguished: genotypic diversity, that is., the number and frequency of multilocus genotypes or clones in a sample of ramets, and genetic diversity, that is, the number and the frequency of alleles (McLellan et al. 1997). Since seedling recruitment is generally infrequent in most clonal plant species (Harper 1977; Eriksson 1993), genetic diversity was supposed to be low in such species (Harper 1977). However, several reviews have shown that genetic diversity can be as high in clonal plant populations as in nonclonal plant populations (Ellstrand and Roose 1987; Hamrick and Godt 1989; Widén et al. 1994). Indeed, Watkinson and Powel (1993) showed that even a small input of successfully establishing seedlings may be sufficient to avoid genetic drift, to prevent the dominance of one or a few clones, and to maintain genetic variation in clonal plant populations.

The spatial structure and distribution of clones is influenced by the efficiency of seed dispersal and the subsequent seed recruitment. The spatial structure of clones is also influenced by the dynamics of the ramets. Lovett Doust (1981) distinguished the phalanx and the guerilla growth forms in clonal plants. In typical phalanx species, the ramets of a

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clone are closely aggregated and clones are juxtaposed. In typical guerilla species, the ramets of a clone are loosely associated and clones can be more intermingled. However, most clonal species have a growth form that falls between these two extreme types.

Among the numerous molecular markers available, the random amplified polymorphic DNA (RAPD) technique has been successfully used to investigate the clonal extent in many plant species (e.g., Bartish et al. 1999; Kreher et al. 2000; Auge et al. 2001; Persson and Gustavsson 2001; Hangelbroek et al. 2002). The reproducibility of RAPD markers, which has been questioned in the literature (e.g., Hedrick 1992), has been largely overcome by improvements in laboratory techniques and band scoring procedures (Nybom and Bartish 2000). In a previous study, we showed that RAPD markers were as effective as amplified fragment length polymorphism (AFLP) markers in detecting clones in *Vaccinium myrtillus* L. (Albert et al. 2003). This has also been shown by Kjølner et al. (2004) for *Saxifraga cernua* L. The comparison of these two types of molecular markers provided an excellent opportunity to check the power of RAPD markers in detecting clones in this species and to confirm the reproducibility of RAPD markers.

In Belgium, three *Vaccinium* species with a shrub or dwarf shrub growth form can be found: *Vaccinium myrtillus* L. (bilberry), *Vaccinium vitis-idaea* L. (lingonberry), and *Vaccinium uliginosum* L. (bog whortleberry). The extent of clonality has previously been investigated in *V. myrtillus* (Albert et al. 2003) and *V. vitis-idaea* (Persson and Gustavsson 2001) but not in *V. uliginosum*. We therefore analysed the clonal structure in natural populations of this species. *Vaccinium uliginosum* and *V. myrtillus* resemble each other in many aspects (deciduous, bell-shaped flowers, pollination by bumblebees, seeds dispersed by birds). However, data on floral and pollination biology showed that *V. uliginosum* is a more selfing species (Jacquemart and Thompson 1996; Jacquemart 2003). According to theoretical predictions (see Charpentier 2002 for a review), the species with the larger clones and the more phalanx growth form are expected to be the more selfing species because of a higher probability of geitonogamy. Consequently we may expect to observe a different clonal structure in *V. uliginosum* compared with that observed in *V. myrtillus* (Albert et al. 2003). *Vaccinium uliginosum* is a perennial shrub, generally found on acid and poorly drained soil types in peaty heaths, bogs, and birch woods. The species is present in all circumboreal regions (Jacquemart 1996). It propagates vegetatively by horizontal subterranean rhizomes (Calmes and Zasada 1982; Jacquemart 1996).

The main purpose of the present study was to determine the diversity of clones and their spatial structure within *V. uliginosum* populations using RAPD markers and to compare the results with the other Belgian *Vaccinium* species.

Materials and methods

Study sites and sampling

Plant material was collected from two natural *V. uliginosum* populations, "Sacrawé" and "Fange aux mochettes" (subsequently referred to as the SA and the FM populations, respectively) in Upper Ardenne, Belgium. The SA population (lat. 50°15′00″N, long. 5°44′22″E, alt. 652 m), the vegetation of which was composed of *Molinia caerulea* (L.) Moench, *Calluna vulgaris* (L.) Hull, *V. uliginosum*, *V. myrtillus, Erica tetralix* L., *Scirpus cespitosus* L., and *Vaccinium oxycoccos* L., is situated in a peaty heath. Several tree species were present in small numbers, including *Pinus sylvestris*, *Salix* × *multinervis*, and *Sorbus aucuparia*. The FM population (lat. 50°13′34″N, 5°40′45″E, alt. 600 m), situated in a ombrotrophic bog, was characterized by a mixture of *Eriophorum vaginatum* L., *Empetrum nigrum* L., *Calluna vulgaris*, *Andromeda polifolia* L., *V. uliginosum, V. myrtillus*, and *V. oxycoccos*. The bog was overgrown by *Betula pubescens* Ehrh. and *Picea abies* (L.) Karst. These two populations were 7 km apart and mainly separated by *Picea abies* plantations and pastures.

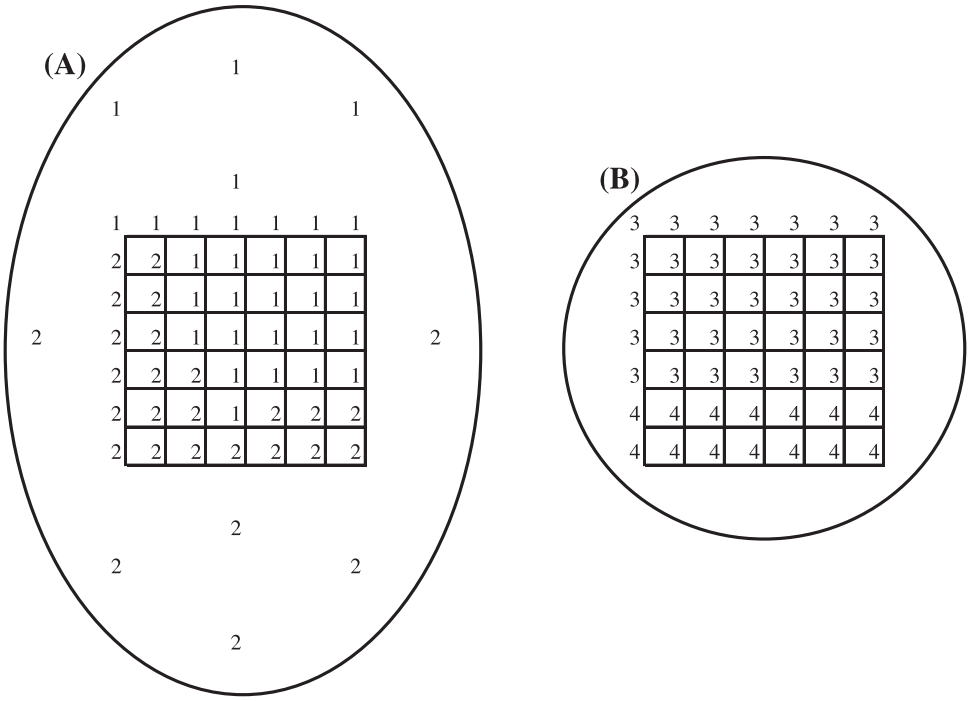
To determine the appropriate sampling scale, a preliminary test was carried out in two homogeneous patches of *V. uliginosum* (referred to as A and B) selected in the SA population. Patches A and B were approximately 45 and 15 m^2 in size, respectively. In both patches, a 3 m × 3 m sampling plot with a 0.5 m × 0.5 m grid was laid out. Within each plot, 49 samples were collected, one at each intersection point. This sampling procedure was previously used for clone identification in *V. myrtillus* populations (Albert et al. 2005). In patch A, which was larger, 10 additional samples were homogeneously collected around the plot (Fig. 1). These 108 samples were analysed by RAPD markers using seven selected primers (see RAPD analysis). Only two clones were identified in each sampled patch (Fig. 1), suggesting that a larger sampling scale was more suitable. Therefore, an area of 90 m × 200 m and one of 60 m × 200 m were arbitrarily selected in the SA and FM populations, respectively. All *V. uliginosum* patches, representing a total of 28 and 19 patches in the selected areas of the SA and FM populations, respectively, were sampled in May 2002. We collected approximately one sample per 5 m^2 in every *V. uliginosum* patch, with samples being evenly distributed within the patches. However, a different sampling scheme was used in the biggest patch of the FM population (Fig. 2*b*) because of its large size (approximately 800 m^2). We sampled intensively only a part of this patch, collecting ramets at each point of intersection of a 15 m × 15 m plot with a 3 m × 3 m grid. Nine additional samples were collected around this plot (Fig. 2*b*). In total, 104 and 115 samples were collected in the 28 SA and 19 FM patches, respectively. The young leaves were stored at –80 °C until DNA isolation. The location of every sampled ramet was mapped by triangulation.

RAPD analysis

DNA was isolated from young leaves using a modification of the cetyltrimethylammonium bromide (CTAB) procedure (Doyle and Doyle 1990). The DNA isolation procedure is given in Albert et al. (2003). The DNA concentration of each sample was estimated by fluorometry using a VersaFluor Fluorometer (Bio-Rad Laboratories, Richmond, California) and samples were diluted to 5 $ng \cdot \mu L^{-1}$ before amplification by polymerase chain reaction (PCR).

All details of the RAPD procedure are given in Albert et al. (2005). PCR products were separated on 1.6% agarose gels (Tris–borate–EDTA buffer), run at 100 V for 195 min,

Fig. 1. Spatial distribution of clones in *Vaccinium uliginosum* patches A and B (delimited by a continuous line). In each 3 m × 3 m sampling plot, samples were collected at each intersection point of a 0.5 m × 0.5 m grid. In the bigger patch, patch A, 10 additional samples were homogeneously collected around the plot. Samples belonging to the same clone are represented by the same number.



stained with ethidium bromide, and visualized under UV transillumination. The presence or absence of DNA fragments was scored using Molecular Analyst Software version 1.12 (Bio-Rad Laboratories 1994).

To determine the best primers in *V. uliginosum*, we analysed four samples, two from each population, and screened these for 107 primers. From this first screening, 14 primers were selected and screened again for polymorphic loci and reproducible banding patterns (using 10 samples, 5 from each population). To test the reproducibility of the RAPD patterns between runs, the 10 samples were amplified twice for each primer. The seven primers yielding the most polymorphism were selected and used for the analysis of the two populations (Table1).

Data analyses

Genetic similarity between pairs of samples was estimated using the Jaccard similarity index (Legendre and Legendre 1998): $S_{xy} = n_{xy}/(n_x + n_y - n_{xy})$, where n_x is the number of bands in sample x, n_y is the number of bands in sample y, and n_{xy} is the number of bands common between samples x and y. A group of samples sharing the same genotype at all polymorphic loci is considered as belonging to the same clone.

To analyse whether there was a spatial genetic structure at the clone level within populations, that is, whether genetic distances observed between clones were correlated with spatial distances between clones, a spatial autocorrelation was performed using the software SPAGEDI version 1.1 (Hardy and Vekemans 2002). The estimator used was Moran's I statistic, which can be computed for each spatial distance class d as follows :

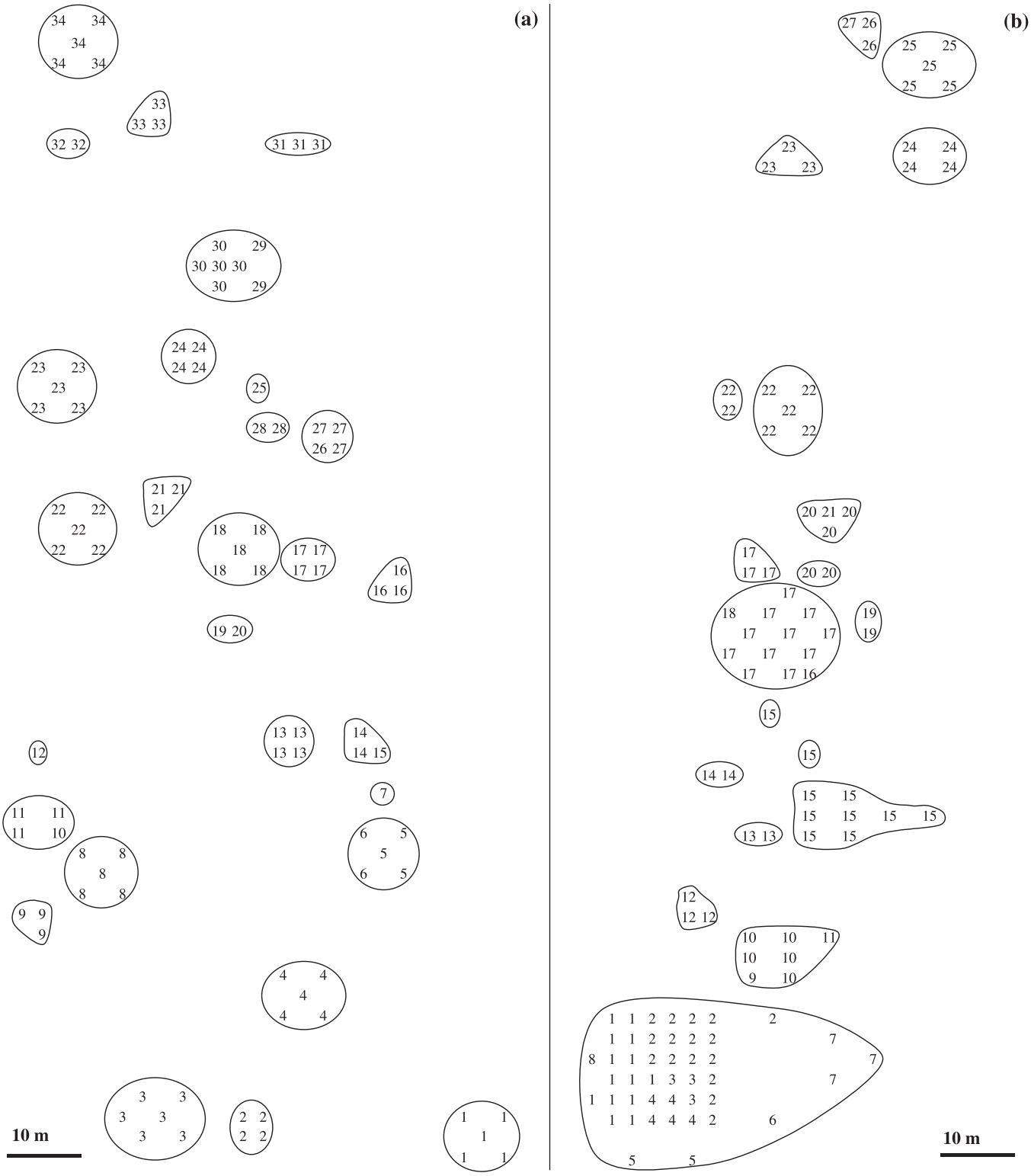
$$I(d) = n\Sigma_i^n \Sigma_j^n w_{ij}(d) \times (x_i - x_m) \times (x_j - x_m)/ [\Sigma_i^n \Sigma_j^n w_{ij}(d)] \times [\Sigma_i^n (x_i - x_m)^2]$$

where n is the number of clones, x_i and x_j are the values of the variable (in this case 1 or 0, depending on the presence or absence of RAPD bands) in clones i and j, respectively, x_m is the mean value of x_i, and $w_{ij}(d)$ is the weight that equals one if the clones i and j are separated by distance class d and otherwise equal zero (Hardy and Vekemans 1999). Duplicate genotypes were removed from the data set so that each clone was represented only once. The distance between clones represented by more than one sample (ramet) was calculated from their average x and y coordinates. Average multi-locus estimators were obtained by averaging the values of $I(d)$ over loci.

To characterize genotypic diversity, three indices were calculated for each plot, following Ellstrand and Roose (1987): (*i*) the number of clones (G) divided by the number of samples (N); (*ii*) Simpson's index corrected for finite sample size (Pielou 1969), $D = 1 - [\Sigma n_i\ (n_i - 1)/N(N - 1)]$, where n_i is the number of samples of genotype i and N is the total number of samples. D ranges from 0 in a population composed of a single clone to 1 in a population where each sample has a unique genotype; (*iii*) genotypic evenness (Fager 1972), $E = (D_{obs} - D_{min})/(D_{max} - D_{min})$, where $D_{min} = [(G - 1)\ (2N - G)]/[N(N - 1)]$ and $D_{max} = [N(G - 1)]/[G(N - 1)]$. E ranges from 0 in a population where each sample has the same genotype or where one genotype dominates and the other genotypes are represented by a single sample to 1 in a population where all genotypes are represented by the same number of samples.

The Shannon's diversity index (Lewontin 1972) was calculated for each RAPD locus, i, as follows: $H_i = -[p_i \log_2 p_i +$

Fig. 2. Spatial distribution of the 34 clones of *Vaccinium uliginosum* in the 28 sampled patches from the SA population (*a*) and the 27 clones in the 19 sampled patches from the FM population (*b*). Every patch is delimited by a continuous line. In each population, samples belonging to the same clone are represented by the same number.



$(1–p_i)\log_2 (1–p_i)]$, where p_i is the frequency of a RAPD band. To obtain the Shannon's diversity index for the population (H_o), H_i was averaged over all loci.

Analysis of molecular variance (AMOVA; Excoffier et al. 1992) was performed to compute the molecular variance components at two levels: within and between populations.

AMOVAs were calculated from a matrix of squared Euclidean distances among all genotypes using Arlequin version 2.000 (Schneider et al. 2000).

Results

The seven selected primers yielded 26 polymorphic markers (Table 1). In the SA population, we were able to identify 34 clones within the 28 sampled patches. Only 6 of the 28 patches consisted of two different clones; the remaining 22 patches (i.e., 79% of the sampled patches) consisted of only one clone (Fig. 2*a*). All clones were restricted to a single patch. In this population, the size of clones averaged 5.7 m (SE = 0.4) and the largest clone, defined as the maximum distance observed between two ramets belonging to the same clone, stretched over 11 m (clone 3). In the FM population, 27 clones were observed within the 19 sampled patches. Fourteen of these patches (74%) consisted of only one clone. A total of eight clones were identified in the biggest patch, with a size of approximately 800 m^2. We detected two to three clones in the remaining four multiclonal patches (Fig. 2*b*). Contrary to what was observed in the SA population, four clones occurred in more than one patch (clones 15, 17, 20, and 22 ; Fig. 2*b*). Two additional primers were used to analyse these four clones and the results confirmed this fragmented structure. Therefore, clones 15 (the largest clone in this population), 17, 20, and 22 stretched over 27, 20, 10, and 15 m, respectively. In this population, the size of clones averaged 9 m (SE = 1.3). None of the genotypes occurred in both the SA and FM populations. The values of the similarity index calculated between clones ranged from 0.37 to 0.94 (mean ± SE: 0.62 ± 0.003) for the SA population and from 0.39 to 0.94 (mean ± SE: 0.65 ± 0.006) for the FM population (mean value for the two populations ± SE: 0.63 ± 0.003).

The spatial autocorrelation analyses among clones indicated an absence of spatial structure in both populations, that is, no correlation was observed between the genetic similarity among clones and spatial distance (SA population: r = –0.001, P = 0.74; FM population: r = –0.011, P = 0.35). These results are consistent with the frequency distributions of the similarity index calculated for pairs of clones within patches and among patches in each population. Indeed, in both populations, the similarity between clones located within any given patch was not higher than the similarity between clones located in different patches (data available upon request). However, because of the low number of intrapatch pair-wise combinations, the similarity indices were not analysed further.

The number of clones (G) divided by the number of samples (N) was 0.33 and 0.23 in the SA and FM populations, respectively (mean: 0.28). The Simpson's index corrected for finite sample size (D) was 0.97 and 0.93 in the SA and FM populations, respectively (mean: 0.95). The genotypic evenness (E) reached 0.98 and 0.93 in the SA and FM populations, respectively (mean: 0.96). Shannon's diversity index (H_o) was 0.663 (SE = 0.063) for the SA population and 0.631 (SE = 0.059) for the FM population (mean = 0.647). Because of the finer sampling scale, lower values of genotypic diversity were obtained for patches A and B (SA population), which were analysed to determine the appropriate sampling procedure (Fig. 1). The values of G/N, D, and E were 0.04, 0.51, and 0.98 for patch A (indices only calculated for the 3 m × 3 m sampling plot), and 0.04, 0.42, and 0.82 for patch B.

Table 1. The number of polymorphic markers from random amplified polymorphic DNA primers for *Vaccinium uliginosum*.

Primer	Sequence	No. of polymorphic bands
A10	5′-GTG-ATC-GCA-G-3′	4
A11	5′-CAA-TCG-CCG-T-3′	3
A15	5′-TTC-CGA-ACC-C-3′	2
UBC35	5′-CCG-GGG-TTA-A-3′	3
UBC52	5′-TTC-CCG-GAG-C-3′	4
UBC78	5′-GAG-CAC-TAG-C-3′	3
UBC84	5′-GGG-CGC-GAG-T-3′	7

According to the AMOVA analysis performed at the clone level, 3.80% of the genetic variability was found between populations and 96.20% within populations (Table 2).

Discussion

Growth form and seedling recruitment

The genetic analysis indicated that *V. uliginosum* was characterized by a typical phalanx growth form, that is, ramets belonging to the same clone tended to be closely aggregated and clones formed distinct clumps (Lovett Doust 1981). This clonal propagation results in population structuring at the ramet level (Figs. 2*a* and 2*b*). In a previous study on *V. myrtillus*, most of the clones also showed a phalanx growth form, although the ramets of some clones were found to be loosely associated and these clones were more intermingled, which corresponded to a more typical guerilla growth form (Albert et al. 2005). *Vaccinium uliginosum* clones were not intermingled. Therefore, along the continuum between typical phalanx and guerilla growth strategies, *V. uliginosum* seems to be located closer to the phalanx end than *V. myrtillus*. On average, the size of clones was higher in *V. uliginosum* (5.7 ± 0.4 m in SA population and 9 ± 1.3 m in FM population) than in *V. myrtillus* (2 ± 0.4 m; Albert et al. 2003). According to the theoretical prediction (see Charpentier 2002 for a review), the species with the larger clones and the more phalanx growth form are expected to be the more selfing species because of a higher probability of geitonogamy. This prediction holds for the *V. uliginosum* and *V. myrtillus* pair. Indeed, *V. uliginosum* is the species that had the larger clones and the more phalanx growth form and was the more selfing species (Jacquemart and Thompson 1996, Jacquemart 2003).

In the present study, most of the *V. uliginosum* patches consisted of a single clone (79% in the SA population and 74% in the FM population). Contrasted results were observed in two previous studies on *V. myrtillus* where all sampled patches consisted of several clones (Albert et al. 2003, Albert et al. 2005) and in one study on *Vaccinium stamineum* where 91% of the patches had more than one clone (Kreher et al. 2000). The higher number of monoclonal

Table 2. Analysis of molecular variance (AMOVA) of RAPD data from the two *Vaccinium uliginosum* populations (SA and FM).

Source of variation	df	Sum of squared deviations	Mean squared deviations	Variance components	% variation
Between populations	1	7.37	7.37	0.13	3.80
Within populations	64	208.69	3.26	3.26	96.20

Note: P values were all significant ($P < 0.0001$). Levels of significance were based on 1000 permutations.

patches observed in *V. uliginosum* suggests that the balance between vegetative reproduction and sexual reproduction is more in favour of vegetative reproduction in this species than in *V. myrtillus* and *V. stamineum*. The higher rate of vegetative reproduction in *V. uliginosum* compared with that of *V. myrtillus* was confirmed by the lower *G*/*N* obtained for *V. uliginosum* (0.04 for both patches A and B in *V. uliginosum*; 0.04 to 0.43 with a mean of 0.17 for 12 patches in *V. myrtillus*, Albert et al. 2005). The above mentioned *G*/*N* values were strictly comparable because the same sampling procedure (plot area and sampling grid) was applied to both cases.

Genetic distances between pairs of clones were not correlated with the spatial distances between these clones. This lack of spatial structure among clones indicates that seedling recruitment events did not occur over a short distance from the mother plant, but that seeds were randomly dispersed in the population. This random seed dispersal in *V. uliginosum* was likely carried out by frugivorous birds (Jacquemart 1996). An absence of spatial structure among clones was also observed in *V. myrtillus* (Albert et al. 2003). Most other studies on population structure in clonal plants obtained the opposite result. This was the case for two other Ericaceae species, *V. stamineum* (Kreher et al. 2000) and *Rhododendron ferrugineum* (Escaravage et al. 1998) as well as for plants from other families, for example, *Galium odoratum* (Ziegenhagen et al. 2003), *Lonicera periclymenum* (Grashof-Bokdam et al. 1998), *Sasa senanensis* (Suyama et al. 2000), and *Viola riviniana* (Auge et al. 2001).

Genetic diversity and partitioning

The mean value of Shannon's diversity index (H_o) for *V. uliginosum* (0.647) was similar to or even higher than those recorded for *V. myrtillus* (0.546; Albert et al. 2003) and for *V. vitis-idaea* (0.568; Persson and Gustavsson 2001). The genetic diversity observed in these three clonal species was comparable to nonclonal species such as *Fitzroya cupressoides* (0.547; Allnutt et al. 1999), *Chaenomeles japonica* and *Chaenomeles speciosa* (0.459 and 0.552, respectively; Bartish et al. 2000). The very low level of seedling recruitment observed in the above-mentioned *Vaccinium* species (Eriksson 1989; Jacquemart 1996; Jacquemart et al. 2003) appears to be sufficient to maintain genetic diversity as high as that in nonclonal species.

Although only two populations were studied, the high proportion of genetic variation within populations revealed by the AMOVA analysis (96.20%) seems to be similar to several RAPD-based AMOVA studies on long-lived woody and herbaceous species with outcrossing breeding systems (genetic variation within populations ranged from 80% to 100%; see Bartish et al. 1999 for a review). A similar proportion of genetic variation (RAPD-based AMOVA) was observed within *V. myrtillus* (86.19%; Albert et al. 2005) and *V. vitis-idaea* (89.2%; Persson and Gustavsson 2001) populations. *Vaccinium uliginosum*, *V. myrtillus*, and *V. vitis-idaea* are long-lived species with mixed breeding systems and seeds dispersed by birds (Ritchie 1955, 1956; Jacquemart 1996; Jacquemart and Thompson 1996). With this combination of life history traits, it is not surprising that these three *Vaccinium* species showed a high proportion of genetic variation within populations and a low proportion of genetic differentiation between populations (see Hamrick and Godt 1996; Bartish et al. 1999; Nybom and Bartish 2000). However, Alsos et al. (2002) obtained a contrasted genetic structure for *V. uliginosum* populations situated in the Arctic Archipelago of Svalbard. Indeed, these authors observed a much higher genetic differentiation among *V. uliginosum* populations ($F_{ST} = 0.493$ from allozyme data) than in most other studied species with similar life history traits (see Hamrick and Godt 1989 for a review). Alsos et al. (2002) hypothesised that this high genetic differentiation between *V. uliginosum* populations was due to infrequent sexual reproduction combined with increased inbreeding and genetic drift resulting from fragmentation events and subsequent bottlenecks during climate cooling.

In summary, we showed that *V. uliginosum* exhibited a typical phalanx growth strategy which resulted in structured populations at the ramet level, that is, ramets belonging to the same clone were closely associated and formed distinct clumps. However, at the clone level, populations were not structured, that is, genetic distances between pairs of clones were not correlated with the spatial distances between the clones within a population. The higher number of monoclonal patches observed in *V. uliginosum* suggests that vegetative reproduction is more important than sexual reproduction in this species compared with *V. myrtillus* and *V. stamineum*. In accordance with the life history traits of *V. uliginosum*, most of the genetic variation was found within populations.

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