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AN ISOZYME STUDY IN BILBERRY (*VACCINIUM MYRTILLUS*) 2. MATING SYSTEM AND GENETIC STRUCTURE

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SUMMARY. — *Vaccinium myrtillus* (bilberry) is an important component of temperate heathland vegetation in Northern Europe, yet little is known concerning basic aspects related to its breeding system and genetic variability. In this study, one metapopulation of bilberry (*Vaccinium myrtillus* L.) was studied in the Upper Ardennes, Belgium, over a period of 3 years in order to estimate outcrossing rates by means of isozyme starch gel electrophoresis. Moreover, this population, along with nine other Belgian populations (in 1992) and three other European populations (in 1993) were sampled for their genetic variability, still by isozyme techniques. The results show that bilberry has a mixed-mating system and levels of genetic variation equivalent to that in other clonal species with sexual reproduction.

RÉSUMÉ. — *Etude isoenzymatique chez la myrtille (*Vaccinium myrtillus*). 2. Système reproducteur et structure génétique. — Vaccinium myrtillus* (la myrtille) est une espèce importante de la végétation des landes tempérées d'Europe du nord. Le système reproducteur et la diversité génétique de cette espèce sont peu connus. Dans cette étude, une métapopulation de myrtilles (*Vaccinium myrtillus* L.) de Haute Ardenne, Belgique, a été suivie durant trois années pour estimer son taux d'allogamie grâce à des électrophorèses d'isozymes en gel d'amidon. D'autre part, 9 populations situées en Belgique (en 1992) et trois autres situées en Europe (en 1993) ont été étudiées quant à leur variabilité génétique, toujours par le biais des isozymes. Les résultats montrent que la myrtille possède un système de reproduction mixte et que le niveau de variation génétique est équivalent à celui des espèces clonales à reproduction sexuée.

1. INTRODUCTION

The potential for evolutionary change is dependent on the existence of genetic variation. As a result, studies of genetic variation in natural populations are a focal point of interest for population and evolutionary biologists (HAMRICK 1989). Studies of the spatial pattern of genetic variation between populations can also provide important insights into aspects of a species' reproductive biology, such as extent and patterns of vegetative reproduction and seed dispersal

(HAMRICK 1982). This is particularly the case for clonal plants for whom the capacity for asexual propagation provides a useful means of examining the effect of mixed reproductive strategies on levels of genetic variation. As pointed out by ELLSTRAND & ROOSE (1987), many clonal plants may maintain relatively high levels of isozyme variation and further research is necessary to elucidate any consequence of clonal reproduction (see recent studies from PARKER & HAMRICK 1992, ASPINWALL & CHRISTIAN 1992, ECKERT & BARRETT 1993).

SCHEMSKE & LANDE (1985) described how a bimodal distribution of mating system (selfed or outcrossed) can be theoretically expected. Further studies have provided evidence that most species do not fit this model (AIDE 1986, HOLSINGER 1988, LYONS & ANTONOVICS 1991). The mating system of a diverse array of plant species needs however to be analysed to provide a realistic picture of the range and norms of plant mating systems (GODT & HAMRICK 1991). Moreover, whilst investigating the genetic variation in natural plant populations and its maintenance, the exact determination of the mating system is of particular importance (BROWN & ALLARD 1970, CLEGG 1980, LYONS & ANTONOVICS 1991, etc.).

Since the late 1960s, allozymes have provided the most abundant source of information regarding genetic diversity and estimations of outcrossing rates in natural populations (BROWN & ALLARD 1970, GOTTLIEB 1981, BROWN & WEIR 1983). This paper is concerned with estimations of outcrossing rates in neighbouring populations of *Vaccinium myrtillus* L. in the Upper Ardennes (Belgium). The report also contains estimates of genetic variability and genetic structure of *Vaccinium myrtillus* at two geographic levels : local (nine populations sampled in southern Belgium) and European (three populations). Starch gel electrophoresis of isozymes provided genetic markers for the estimations of both outcrossing rates and genetic variability.

Vaccinium myrtillus, the bilberry, is a diploid perennial rhizomatous shrub found in a variety of temperate woodlands and heaths. It is widely encountered from Northern to Central Europe. Flowers bloom in the early spring as the leaves expand. These flowers are predominantly visited by bumblebee queens and syrphids (JACQUEMART 1992a). Experiments have shown that the bilberry is a hermaphroditic self-compatible species (JACQUEMART in prep.). Nevertheless, its clonal habit and the rarity of seedlings in nature lead to the conclusion that vegetative reproduction is considerably more important than recruitement by seed (RITCHIE 1956).

2. MATERIAL AND METHODS

2.1. MATING SYSTEM INVESTIGATION

For the estimation of outcrossing rates, genotypic distributions in progenies constituted the data base. Berries were collected from nine to thirty families (individuals) per population and set to germinate in petri dishes on a synthetic moss layer covered with filter paper (16 hour day-lenght, 15-25°C diurnal temperature). The petri dishes were wrapped in plastic film in order to avoid water loss by evaporation. Once first leaves had developed, seedlings were transplanted to acid soil plots. Eight to twenty-five offspring per family were assayed for isozyme genotype at two or three loci (*Mdh*-3, *Tpi*-1 and *Mnr*-1, the most polymorphic loci revealed). During three years, neighbouring populations from the Upper Ardennes (Belgium), were assayed in a heathland at Sacrawé in 1990 and 1991 (two subpopulations in 1991) and in a peaty wood at Robièfa in 1992.

Horizontal electrophoresis was conducted on 12.5 per cent starch gels on enzyme extractions from seedlings having developed at least eight leaves. Extracts were made using an extraction buffer consisting of 0,1 M Phosphate buffer (pH 7.5) with 10,0 per cent (w/v) PVP-40 and 1,0 per cent (v/v) 2-mercaptoethanol. Two different buffer systems were used : (A) Morpholine-Citrate pH 6.1 and (B) Histidine-HCl pH 6.5. Electrophoresis and enzyme staining methods generally followed a similar pattern as those described in JACQUEMART (1992b).

The data were analysed using the multilocus outcrossing estimate program MLT (RITLAND 1990). The single and multilocus outcrossing rates (t) represent the proportion of progeny that result from an outcrossing event, 1.00 being the maximum value. Significant deviations from t=1 were tested using the test-statistic (t-t0)/var(t), which follows (asymptotically) a Chi-square distribution with one degree of freedom (RAO 1973

TABLE 1

Population and study descriptions

Country	Population name (locality)	Long. Lat.	Habitat	Study
High-Ardenne Belgium	*Sacrawé (Bihain-Vielsalm)*	*50°15'N 5°44"E*	Heathland	1989 : **MS** 1990 : **MS**
	Wé-des-Pourceaux (Bihain-Vielsalm)	*50°15'N 5°42"E*	Peaty Wood	1991 : **MS** 1992 : *BGSm*
	Robièfa (Odeigne)	*50°13'N 5°47"E*	Peaty Wood	1992 : **MS** 1992 : *BGSm*
	Pisserotte (Houffalize)	*50°15'N 5°44"E*	Heathland	1992 : *BGSm*
	Thier Du Mont (Sart-Lierneux)	*50°16'N 5°52"E*	Heathland	1992 : *BGSm*
Ardenne Belgium	*Bois de St-Hubert (Saint-Hubert)*	*50°04'N 4°20"E*	Beech groove	1992 : *BGSm*
	Bois de St-Michel (Saint-Hubert)	*50°04'N 4°20"E*	Beech groove	" "
	Mochamps (Saint-Hubert)	*50°05'N 4°21"E*	Beech groove	" "
	Bois de Nassogne (Nassogne)	*50°05'N 4°21"E*	Beech groove	" "
Central Belgium	*Bois de Lauzelle (Ottignies)*	*50°40'N 4°35"E*	Pine wood	1992 : *BGSm*
	Les Bruyères (Chaumont-Gistoux)	*50°40'N 4°44"E*	Pine wood	1992 : *EGSs*
Finland	*(Oulu)*	*65°00'N 25°30"E*	Pine wood + Peaty heath.	1992 : *EGSs*
Switzerland	*(Mathon)*	*46°39'N 9°24"E*	Mineral heathland	1992 : *EGSs*

MS : estimation of outcrossing rate ; *BGSm* : genetic structure from mother, Belgian level ; *EGSs* : Genetic structure from seedling, European level.

cited by KONIUSZEK *et al.* 1986). From the progeny genotypes Wright fixation coefficient was calculated as : (1) $F = 1-(h/(1-\Sigma p_i^2))$, where h is the observed frequency of heterozygotes, and p_i is the frequency of the ith allele. The expected inbreeding coefficient at equilibrium was calculated from the multilocus outcrossing rates by the equation : (2) $Fe = (1-t)/(1+t)$.

2.2. GENETIC STRUCTURE OF NATURAL POPULATIONS

In the first analysis, an average of fifteen plants per population were sampled in nine populations from Belgium in March 1992 (for population descriptions, see Table 1). Ramets were grown in a greenhouse until vegetative buds emerged.

In a second experiment, in July 1992, berries were collected separately from fifteen individual plants ; one population originating from Belgium (Chaumont-Gistoux), one from Switzerland (Mathon) and one from Finland (Oulu) (Table 1). Twenty-six offspring from a total of six mothers were assayed per population, except for Lap enzyme (16-20 offspring) and for Pgm enzyme (6-12 offspring).

Horizontal electrophoresis was conducted in 12.5 per cent starch gels on enzyme extracted from emerging vegetative buds (first experiment) or from seedlings having developed at least eight leaves (second experiment).

The following gene diversity statistics were obtained using the microcomputer version of BIOSYS (SWOFFORD & SELANDER 1981) : **K**, mean number of alleles per locus, **PLP**, percentage of polymorphic isozyme loci (i.e. which have more than one allozyme), **H**, within population heterozygosity (expected assuming Hardy-Weinberg equilibrium) averaged over all loci, and **I** (NEI 1973), unbiased standard genetic identity (similarity in allozyme frequencies between populations) for pairs of populations. The other indices were computed as indicated by NEI (1973) : $\mathbf{H_S}$, mean of all H's, $\mathbf{H_T}$, heterozygosity within this whole group of individuals (expected - as above) mean of all loci, $\mathbf{G_{ST}}$, proportion of total heterozygosity that is due to population differentiation $((H_T - H_S)/H_T)$.

3. RESULTS

3.1. OUTCROSSING RATES

Estimations of the single and multi-locus outcrossing rates in each of the populations are shown in Table 2. Mean single-locus estimates of outcrossing for each population ranged from 0.57 to 0.71, with an overall mean of 0.62. There were significant differences among single-locus estimates, *Mdh*-3 showing smaller outcrossing rates than *Tpi*-1 (Table 2). Multilocus estimations of outcrossing rates ranged from 0.66 to 0.75, with an averaged population value of 0.72. Three of the estimations were significantly different from 1. Multilocus outcrossing estimates were quite similar between populations and from year to year. There was a disparity between the multilocus outcrossing rates and the mean single-locus estimates, which were smaller in all populations.

The observed inbreeding coefficients (Fo) were generally positive except for the Wé-des-Pourceaux population (-0.058) (Table 3). The equilibrium inbreeding coefficients (Fe) estimated from the multilocus outcrossing rates were positive. Their value ranged from 0.143 to 0.205. The equilibrium coefficient was greater than that observed in each population, except Robièfa.

3.2. GENETIC VARIABILITY

Of the ten loci revealed in the two studies, six (*Idh*-1, *Lap*-1, *Mdh*-3, *Pgi*-2, *6-Pgd*-2, *Tpi*-1) were polymorphic in at least one population (Table 4) and six were common to the two studies

TABLE 2

Outcrossing rates (S.E.)

	Upper Ardennes (Belgium)				
	Sacrawé		Wé-des-Pourceaux	Robièfa	Mean population
Year	1990	1991	1991	1992	
t_s *Mdh-3*	0,57	0,34	0,41	0,18	
	(0,12)	(0,14)	(0,15)	(—)	
t_s *Tpi-1*	0,75	0,84	0,80	0,96	
	(0,15)	(0,18)	(0,09)	(—)	
t_s *Mnr-1*	0,54	0,54	—	—	
	(0,12)	(0,10)			
mean t_s	0,71	0,59	0,60	0,57	0,62
	(0,09)	(0,10)	(0,12)	(—)	
	**	***	***	***	
t_m	0,75	0,66	0,73	0,75	0,72
	(0,10)	(0,08)	(0,12)	(0,21)	
	**	***	*	NS	

t_s : single-locus outcrossing rate ; mean t_s : single locus outcrossing rate averaged over all loci ; t_m : multilocus outcrossing rate, different from one at * $P < 0,05$, ** $P < 0,02$, *** $P < 0,001$.

(*Mdh*-2, *Mdh*-3, *Pgi*-2, *Pgm*-2, *6-Pgd*-2, *Tpi*-1). Two loci considered as monomorphic by JACQUEMART (1992b) showed polymorphism in at least one population (*6Pgd*-2 in the Bois de Lauzelle (Ottignies) population and *Pgi*-1 in the European populations). These loci are now interpreted as diallelic coding for dimeric enzymes. Furthermore, the Oulu population (Northern Finland) exhibited a third allele at *Mdh*-3 locus. This locus is now interpreted as triallelic coding for a dimeric enzyme.

Genetic diversity estimates were then calculated from the eight loci revealed in each study and for the six loci in common. Estimations of genetic variability within populations are given in Table 5 as estimates averaged over populations of the percentage of polymorphic loci (PLP), the mean number of alleles per locus (K), the mean expected heterozygosity (H_m) and the mean observed heterozygosity (H_o). When comparing the heterozygosity (observed as well as expected) obtained in each study, the difference between the two sets of populations is greater when all the loci are considered in each study than when only the six common loci are taken into account (i.e. *Mdh*-2, *Mdh*-3, *Pgi*-2, *Pgm*-2, *6-Pgd*-2, *Tpi*-1). This can be explained by the differences in the two sets of loci. *Lap*-1 was revealed only in European samples where this locus accounted for 42,8% of Hm. There was no such polymorphic locus revealed in the Belgian populations. This emphasizes the potentially great influence of a single locus on the estimation of heterozygosity and therefore, the necessity of sampling as many

TABLE 3

Fixation indices

Population	(Year)	Fo	Fe	ΔF	t_m
Sacrawé	(1990)	0,108	0,143	-0,035	0,75
	(1991)	0,163	0,205	-0,042	0,66
Wé-des-Pourceaux	(1991)	-0,058	0,156	-0,214	0,73
Robièfa	(1992)	0,151	0,143	0,008	0,75

Averaged observed values of Wright's fixation index (Fo) in seedling population based on two loci (*Mdh-3 and Tpi-1*) ; expected values of Wright's fixation index (Fe) based on estimated outcrossing rate (t_m) and averaged ΔF values (Fo-Fe) for *Vaccinium myrtillus* in the Upper Ardennes.

TABLE 4

Polymorphism of the enzymes analysed in the two studies

Enzyme	Locus	Belgium	Europe
Aldolase	*Ald-2*	—	M
Isocitrate dehydrogenase	*Idh-1*	P-2	—
Leucine amino peptidase	*Lap-1*	—	P-3
Malate dehydrogenase	*Mdh-2*	M	M
	Mdh-3	P-2	P-3
Menadione reductase	*Mnr-2*	M	—
6-Phosphogluconate dehydrogenase	*6-Pgd-2*	P-2	M
Phosphoglucoisomerase	*Pgi-2*	M	P-2
Phosphoglucomutase	*Pgm-2*	M	M
Triose phosphate isomerase	*Tpi-1*	P-2	P-2

P = Polymorphic locus ; M = Monomorphic locus ; the number of alleles is indicated directly after the status ; "—" = this locus is not analysed in this study.

loci as possible. Thus, we can assume that there is no significant difference between Belgian and European populations concerning the genetic variability within populations.

As expected, there is a more marked difference between the two geographical areas when considering the gene diversity at the species level (see Table 6). Whether all the loci or only the common ones are taken into account, the gene diversity (H_T) is, not surprisingly, greater on the European scale than in Belgium alone (0.174 and 0.075 for eight loci and 0.137 and 0.098 for the six loci in common). Accordingly, the values of D_{ST} and G_{ST} obtained at the European level are higher ($D_{ST} = 0.004$ and 0.022, $G_{ST} = 0.056$ and 0.127 at the Belgian and European level respectively, for eight loci and $D_{ST} = 0.006$ and 0.019, $G_{ST} = 0.061$ and 0.139 for the six common loci). This emphasizes the importance of sampling over a sufficiently wide geographical range when the genetic variability of a species as a whole is of interest. However, the distinction between the two geographic ranges is less clear-cut if Nei's gene identities (Table 6) are taken into account, because the standard errors are relatively high in the European study (S.E. = 0.017). This is at least partly due to the small number of populations sampled (three) at the European level.

TABLE 5

Genetic variability within populations of Vaccinium myrtillus *L.*

Populations	(year)	N pop.	N loci	K (S.E.)	PLP (S.E.)	H_m (S.E.)
A. Based on all loci revealed						
Belgium	(1992)	9	8	1.26 (0.04)	20.8 (8)	0.071 (0.021)
Europe	(1992)	3	8	1.63 (0.00)	37.5 (0.00)	0.155 (0.037)
B. Based on the 6 loci in common						
Belgium	(1992)	9	6	1.30 (0.00)	27.8 (8.3)	0.092 (0.028)
Europe	(1992)	3	6	1.50 (0.00)	33.3 (0.00)	0.118 (0.030)

Mean number of alleles per locus (K) ; percentage of polymorphic loci, 0.95 criterion (PLP) ; mean expected heterozygosity (H_m).

TABLE 6

Gene diversity of Vaccinium myrtillus *at species level*

Populations	(year)	N pop.	N loci	H_T	H_S	D_{ST}	G_{ST}	$\bar{I}$ (S.E.)
A. Based on all revealed loci								
Belgium	(1992)	9	8	0.075	0.071	0.004	0.056	0.994 (0.008)
Europe	(1992)	3	8	0.174	0.152	0.022	0.127	0.967 (0.017)
B. Based on the 6 loci in common								
Belgium	(1992)	9	6	0.098	0.092	0.006	0.061	0.992 (0.011)
Europe	(1992)	3	6	0.137	0.118	0.019	0.139	0.967 (0.017)

Measures of Nei's gene diversity (1973, 1986) averaged over all polymorphic and monomorphic loci and averaged Nei's unbiaised gene identity ($\bar{I}$) (Nei, 1978) in *Vaccinium myrtillus* L.

4. DISCUSSION

4.1. Breeding system

With an intermediate outcrossing rate, *V. myrtillus* can be classified as mixed mating (t = 0.61-0.80, *sensu* Schemscke & Lande 1985). Other studies of mating systems show that partial selfing occurs in animal-pollinated species (Brown *et al.* 1985). The moderately high rate of selfing (1 - mean t_m = 28%) in bilberry populations is consistent with the bumblebee foraging behaviour which geitonogamy is likely to be particularly high. The intermediate level of outcrossing in bilberry does not conform to the bimodal distribution of very high or very low outcrossing rates predicted by Schemske & Lande (1985), as observed in many other species (Holsinger 1988, Lyons & Antonovics 1991).

Variations between single-locus estimates have regularly been reported (Sanders & Hamrick 1980, Krueger & Knapp 1991, Schwarzmann & Gerhold 1991). This variation could be due to selection acting differentially on loci linked to different allozyme marker loci (Brown & Allard 1970, Nevo *et al.* 1992). Heterogeneity among these estimates may also have been caused by spatial genetic structure of population (Sanders & Hamrick 1980) or different allele frequencies and levels of polymorphism for each locus (Ritland & Jain 1981, Brown *et al.* 1985, 1989). However, variation could occur as a sampling effect especially when the number of maternal plants is restricted (Brown *et al.* 1985). Such an explanation could be the case for the Wé-des-Pourceaux population (nine families) and for the Robièfa population (thirteen families).

Multilocus measures of outcrossing rates are generally considered more accurate estimates of the mating system because of their hardiness to violations of the assumptions of the mixed mating model (Ritland & Jain 1981, Shaw *et al.* 1981). Differences between mean single-locus and multilocus outcrossing estimates are commonly attributed to biparental inbreeding, i.e. outcrossing between relatives (for details see Shaw *et al.* 1981 and Shaw & Allard 1982). For this study, the mean multilocus estimate of selfing was 28% when the mean single-locus estimate was 38%. On average, 26% [(38-28)/38] of apparent selfing in the population was due to biparental inbreeding. *Vaccinium myrtillus* berries, if not dispersed by birds, fall in mother proximity. This gravity dispersal leads to a population substructure consisting of related individuals. Limited seed dispersal makes it likely that biparental inbreeding contributes significantly to the inbreeding experienced by many plant populations (Godt & Hamrick 1991). Otherwise, selfing could be favorised : bilberry reproduces mainly by rhizome sprouting, so vegetative spreading could significantly increase the number of geitono-pollinations.

As several reviewers have stressed (Hamrick 1982, Schemske & Lande 1985), outcrossing rates can vary at all levels of organization within a species. There may be considerable variation in outcrossing among plants within populations, due to the presence of genetic structuration within the population (Hamrick 1982) and due to the presence of sexual polymorphism as gynodioecy (Sun & Ganders 1986). Thus, high standard errors observed in this study for multilocus estimates (0,08 to 0,21) are not surprising and can be due to internal population variation rather than sampling effects. Many of the animal-pollinated species showed high interpopulation variation that is attributed to pollinator availability, population size, density, floral structure and degree of self-compatibility (Aide 1986, Brown *et al.* 1989 ; Godt & Hamrick 1991). The lack of such heterogeneity between bilberry populations sampled many times could be due to the tolerance of bumblebee pollinators to variation of climatic conditions. In this way, the bumblebee visitation rates and consequently the outcrossing rates could be similar for one population sampled over several years.

Both observed and expected fixation indices showed unusually higher heterozygote frequencies (negative ΔF). It has been previously demonstrated that the distribution of heterozygotes frequently exceeds that expected on the basis of the mating system alone (Clegg 1980). In addition, indirect evidence in this study suggests that heterozygote selection occurs between the seed and adult stage of the life cycle. Genotypes at

two loci, *Tpi*-1 and *Mdh*-3, were determined for seedlings and parental vegetative buds in 1990 for the population of Sacrawé (Upper Ardennes). F values were smaller for the adult genotypes than for the seedlings (F for seedlings were -0.038 and 0.082 for *Tpi*-1 and *Mdh*-3 respectively and F for adults were -0.422 and -0.349 respectively), i.e. adult heterozygote frequencies exceeded those of juveniles. Since one of the cost of self-pollination is assumed to be decreased survivorship of individuals homozygous at loci undergoing selection from heterozygotes, this finding is relevant to cost-benefit models of the evolution of autogamy (SCHOEN 1982).

4.2. GENETIC VARIABILITY

Several studies have demonstrated a statistical relationship between life history characteristics and enzymatic variability of diploid species (GOTTLIEB 1981, LOVELESS & HAMRICK 1984, HAMRICK & GODT 1989). The relationship between breeding system and genetic structure has been addressed in all these studies. The values obtained for several genetic population parameters (based on the six common loci) both at European and Belgian levels are similar to the values found for species with mixed animal breeding, i.e. PLP = 29.2 and $H_m = 0.09$ (HAMRICK 1989). Analysis at the species level shows that most genetic variation in bilberry occurred within populations and that variation among populations is only responsible for a maximum of fourteen per cent of the total diversity ($G_{ST} = 0.139$). This pattern corresponds to predictions based solely on breeding behaviour revealed by outcrossing rate (GOTTLIEB 1981, HAMRICK 1989).

Table 7 provides a comparison of genetic variability between *Vaccinium myrtillus* and three diploid American species of *Vaccinium* studied by BRUEDERLE *et al.* (1991). Differences in the partitioning of variation among the diploid blueberries would seem to be due to differences in life history traits (habit, geographic distribution, ecology, mating system). The comparison of estimations of the absolute genetic variability is somewhat hindered by the differences between the two studies, especially regard to the loci analysed. It is reasonable however, to assume that H_T and H_S are roughly the same in *V. myrtillus* and the two American species *V. elliottii* and *V. myrtilloides*. The values obtained for Nei's minimum genetic distance ($D_m = 0.029$ to 0.033) are closer to the pair *V. myrtilloides* - *V. tenellum* (Dm = 0.029 to 0.030). However, it appears that the structuration of the genetic variability is quite different in *V. myrtillus* compared to the three American species. Indeed, the proportion of the variability among populations relative to the variability within populations ($\mathbf{R_{ST} = D_m / H_S}$) is greater in *V. myrtillus* (0.242 for *V. myrtillus* vs. 0.168 to 0.188 for American *Vaccinium*). The American species, which is roughly the closest to *V. myrtillus* regard to the extent and organisation of genetic variability, is *V. myrtilloides*. Interestingly enough, *V. myrtilloides* is precisely the species which presents the most similar life history traits to *V. myrtillus* (rhizomatous low-bush, widespread, uniform distribution at a site, entomophilous).

V. myrtillus, a highly rhizomatous shrub, showed both at population and species level, roughly the same level of genetic diversity as *V. elliottii*, a congeneric crown forming shrub

TABLE 7

Comparison of the genetic diversity at the species level between Vaccinium myrtillus *L. and three American* Vaccinium *species* (BRUEDERLE *et al.*, 1991)

Species	N pop.	N loci	H_T	H_S	G_{ST}	D_m	R_{ST}
V. myrtillus	3	8	0.174	0.152	0.127	0.033	0.217
V. myrtillus	3	6	0.137	0.118	0.139	0.029	0.242
V. elliottii	4	20	0.134	0.119	0.126	0.020	0.168
V. myrtilloides	5	20	0.176	0.153	0.133	0.029	0.188
V. tenellum	5	20	0.191	0.167	0.127	0.030	0.180

($H_T = 0.174$ and 0.134 and $H_S = 0.152$ and 0.119 respectively). Our results corroborate the observations of ELLSTRAND & ROOSE (1987) and HAMRICK & GODT (1989), i.e., that predominantly clonal species, as *Vaccinium myrtillus*, may maintain as much genetic diversity within populations as purely sexually reproducing species.

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