Floral and pollination biology of three sympatric *Vaccinium* (Ericaceae) species in the Upper Ardennes, Belgium

A.-L. Jacquemart and J.D. Thompson

Abstract: Comparative studies of the reproductive biology and pollination ecology of closely related species allow us to test several ideas related to the evolution of selfing taxa from outcrossing ancestors. The existence of closely related species in the same habitat provides a particularly useful opportunity to examine this issue. A variety of floral traits likely to be associated with the reproductive system of three sympatric *Vaccinium* species (*V. myrtillus*, *V. vitis-idaea*, and *V. uliginosum*) were quantified in a heathland in the Upper Ardennes, Belgium. These traits included the length and width of the corolla, the number and size of the anthers, the number of pollen tetrads and ovules, and the length of the style. Pollen to ovule ratios suggest a mixed mating system in the three species. The greater pollen to ovule ratio and stigma–anther separation in *V. vitis-idaea* suggest that it functions more as an outcrosser than the two congeners. The effects of caging, emasculation, and artificial pollination on fruit and seed set differed among years and among the three species. Supplementary pollination increased fruit set and fruit characteristics (particularly seed number) relative to natural pollination in the three species. The three species showed a varied but poor capacity to self in the absence of pollinators. Seed set per fruit was lower in the spontaneously selfed flowers in comparison with hand-crossed pollinated flowers in *V. myrtillus* and *V. vitis-idaea* but not in *V. uliginosum*. This higher ability to self in *V. uliginosum* indicates a lower capacity to self in the absence of pollinators. However, all the three species were at least partially self-compatible. Together the floral traits and selfing ability suggest that the polyploid *V. uliginosum* appears to be more highly selfing than the two diploids, particularly *V. vitis-idaea*.

Key words: floral biology, mixed mating, mating system, *Vaccinium*, seed set.

Résumé : Des études comparatives en biologie de la reproduction et en écologie de la pollinisation concernant des espèces apparentées nous permettent de tester plusieurs idées relatives à l'évolution des taxons autogames à partir d'ancêtres allogames. L'existence d'espèces très proches occupant un même habitat fournit une occasion particulièrement intéressante d'examiner cette question. Un certain nombre de traits floraux susceptibles d'être associés au système reproducteur de trois espèces sympatriques de *Vaccinium* (*V. myrtillus*, *V. vitis-idaea* et *V. uliginosum*) ont été mesurés dans une lande de haute Ardenne, en Belgique. Ces traits comprennent la longueur et la largeur de la corolle, le nombre et la taille des anthères, le nombre de tétrades de pollen et le nombre d'ovules ainsi que la longueur du style. Les rapports pollen à ovule obtenus suggèrent l'existence d'un système reproducteur mixte chez les trois espèces. Le rapport pollen à ovule plus élevé ainsi que la plus grande séparation stigmate–anthère chez *V. vitis-idaea* laisse penser que cette espèce fonctionne d'une manière plus allogame que ces deux congénères. Les effets de l'ensachement, de l'émasculation et de la pollinisation artificielle sur la production de fruits et de graines diffèrent selon les années et les espèces. Le supplément de pollen augmente la production de fruits et les caractéristiques de ces derniers (particulièrement le nombre de graines) chez les trois espèces. Les trois espèces présentent une faible capacité à s'auto-polliniser spontanément en l'absence de pollinisateurs. La production de graines est plus faible chez les fleurs auto-pollinisées spontanément que chez les fleurs pollinisées par du pollen allogame chez *V. myrtillus* et *V. vitis-idaea* mais non chez *V. uliginosum*. Cette plus grande possibilité de s'autoféconder chez *V. uliginosum* va de pair avec une capacité plus faible à s'autopolliniser en l'absence de pollinisateurs. Les trois espèces sont cependant au moins partiellement auto-compatibles. Les traits floraux tout comme
la capacité à s'autoféconder suggèrent que l'espèce polyploide, *V. uliginosum*, serait plus autogame que les deux diploides, et plus particulièrement par rapport à *V. vitis-idaea*.

Mots clés : biologie florale, système reproducteur, système mixte de reproduction, *Vaccinium*, production de graines.

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Introduction

The comparison of self- and cross-pollination in relation to the functional aspects of floral biology and pollination relationships (Barrett and Eckert 1990; Lloyd and Schoen 1992; Schoen and Lloyd 1992) and evolutionary changes in levels of genetic load and inbreeding depression (Uyenoyama 1986) represents a central concern of plant reproductive biology. For instance, variation in floral characteristics that either promote cross-fertilization or enable a species to self-pollinate in the absence of pollinators represent essential components of mating system evolution. Outcrossing plants are often protandrous, with marked stigma–anther separation, large showy flowers that have relatively long blooming times, and high pollen to ovule ratios. In contrast, self-fertilizing taxa often show reduced temporal and spatial separation of male and female function, flower size and attractiveness, flowering duration, and pollen to ovule ratios (Lloyd and Webb 1977; Schoen 1982*a*; Wyatt 1988; Cruden and Lyon 1989; Ramsey 1993).

Although intraspecific variation sets the scene for microevolution in mating systems, patterns of variation in closely related taxa provide a means to examine how variation in floral characteristics depend on and may contribute to mating system variation (Cruden and Lyon 1985; Ritland and Ritland 1989; Mazer and Hultgard 1993). Furthermore, comparison of floral variation among closely related species and the effect of such variation on the mating system could be of particular importance to comparative studies of the reproductive biology of closely related taxa and the evolution of mating systems (Schemske 1983; Ritland and Ritland 1989; Dole 1992; Mazer and Hultgard 1993; Pickering and Ash 1993).

Despite the large amount of work contained in the above studies, there has been little attention given to the range of closely related species inhabiting peat bogs and wet heathland communities. In such communities, the Ericaceae family is a very significant element and four genera (*Andromeda, Calluna, Erica*, and *Vaccinium*) constitute the major components of the perennial shrubs. This study is part of ongoing research into the pollination ecology and mating-system variation among closely related heathland plants in western Europe. In this paper, we focus our attention on three European *Vaccinium* species: *V. myrtillus*, the bilberry; *V. vitis-idaea*, the cowberry; and *V. uliginosum*, the bog whortleberry. These three species occur in sympatry in various sites on peaty heath at moderately high latitude and (or) altitude in Europe. Thus they represent a useful system to examine how variation in floral traits may be associated with the reproductive biology of different species.

Although the floral morphology of *Vaccinium* species was well documented by Warming (1908), their breeding systems are unknown. Ritchie (1955*a*, 1955*b*, 1956) studied *V. myrtillus, V. vitis-idaea*, and their hybrid *V.* ×*intermedium* and concluded that their flowers are both outcrossed by insects and self-pollinated, with the former being more common. The aim of this study was to document the floral pollination reproductive biology of these three species in a heathland community in the Upper Ardennes, Belgium, where they are sympatric. We focussed on variation in floral traits that is related to the ability of the species to self and their relative fecundity on selfing and outcrossing.

We asked four questions. (*i*) Do the three species differ significantly in floral traits? (*ii*) Is variation in floral traits among species due to differences between the tetraploid *V. uliginosum* and the two diploid species? (*iii*) Does variation in floral traits correlate with the ability to self in the absence of pollinators and the self-compatibility of the three species? (*iv*) Do the species show evidence for pollen limitation on fruit and seed sets?

Materials and methods

Study species

The genus *Vaccinium* L. (Vaccinoidae, Ericaceae), largely holarctic in its distribution, consists of about 450 perennial species of small trees and shrubs with tetra- or penta-meric flowers and fruit in the form of an edible berry (Cane et al. 1985). The anthers of *Vaccinium* spp. have terminal, poricidal dehiscence and only *V. vitis-idaea* is awnless (Warming 1908; Matthews and Knox 1926; Ritchie 1955*a*, 1955*b*, 1956). Reproduction is both vegetative and by seeds. Plants spread vegetatively by rhizomatous clonal growth, often forming dense clones (Ritchie 1955*a*, 1955*b*, 1956; Flowers-Ellis 1971). Flowers are nectariferous, attracting a wide array of pollinators and nectar thieves (Jacquemart 1992, 1993*a*). The biology of the three species can be summarized as follows.

Vaccinium myrtillus (bilberry) is a deciduous, diploid ($2n = 24$; Hagerup 1928; Uotila and Pellinen 1985), clonal dwarf shrub (10–60 cm) with an extensive rhizome system. The flowers are single or rarely in pairs in the leaf axils and present green to purple pendulous urceolate corollas (Ritchie 1956). *Vaccinium myrtillus* has a paleoarctic distribution and occurs up to 2800 m in altitude (Ritchie 1956).

Vaccinium vitis-idaea (cowberry) is an evergreen diploid heath shrub (Hagerup 1928; Ritchie 1955*a*; Uotila and Pellinen 1985). It has a slightly pendulous raceme of 2–12 campanulate white flowers. It is smaller in height (10–30 cm) than *V. myrtillus*. *Vaccinium vitis-idaea* has an arctic-alpine distribution (Gimingham 1972).

Vaccinium uliginosum spp. *uliginosum* (whortleberry) is deciduous and tetraploid (Rousi 1967; Uotila and Pellinen 1985). It has greenish white pendulous, urceolate, globose flowers (Warming 1908). The inflorescences bear one to four flowers grouped in subterminal pseudo-ombellates. This species is larger (40–80 cm) than the previous two species and has an arctic-alpine distribution but at higher altitudes and latitudes than *V. vitis-idaea* (Stewart and Bannister 1973).

Vaccinium myrtillus is the first species to flower each year, followed by *V. uliginosum* and then by *V. vitis-idaea* (see Jacquemart 1993*a* for details). Taxonomically, *V. myrtillus* and *V. uliginosum* are classified in the *Myrtillus* Koch section and *V. vitis-idaea* in *Vitis-idaea* (Moench.) Koch section of the genus (Ritchie 1955*a*, 1956; Clapham et al. 1962).

Study site

The study was carried out over 3 consecutive years, from 1988 to 1990, at a field site in the Upper Ardennes, Belgium (50°15′00″N, 5°44′22″E, alt. 652 m). The site consists of a heathland and a peat bog dominated by several ericaceous plants: the four European *Vaccinium* species are present (the three species studied plus *V. oxycoccos*) as well as *Calluna vulgaris, Erica tetralix*, and *Andromeda polifolia*. In the forested area, *Sorbus aucuparia, Frangula alnus, Salix* ×*multinervis*, and *Pinus sylvestris* are the most common trees. The heath is bounded by *Picea* plantations and meadows. Collections and field experiments extended from early April until late August in each year.

Table 1. Description of the nine treatments used in the pollination trials on three *Vaccinium* species in the high Ardennes.

Treatment code	Treatment	Effect measured	Specific type of selfing	Specific type of outcrossing	Year
T1	Free exposure	Natural fruit set	Natural	Natural	1988, 1989, 1990
T2	Supplemental cross-pollination	Pollen transfer limitation	Natural	Natural + artificial	1989, 1990
T3	Emasculation, cross-pollination	Emasculation + bag effects	No	Natural + artificial	1990
T4	Emasculation, no pollination	Reproductive assurance	No	Natural	1989, 1990
T5	Bagged, emasculation, self-pollination	Allogamy	No	Artificial	1988, 1989, 1990
T6	Bagged, emasculation, self-pollination	Geitonogamy	Natural + artificial	No	1989, 1990
T7	Bagged, no emasculation, self-pollination	Autogamy	Artificial	No	1988, 1989, 1990
T8	Bagged, emasculation, no pollination	Apomixis	No	No	1990
T9	Bagged alone	Spontaneous autogamy	Natural	No	1988, 1989, 1990

Experimental study

Floral morphology

Twelve floral buds of each species were collected immediately prior to anthesis and were preserved in formalin – acetic acid – ethanol 60% (FAA; 1:1:8) for pollen counts. For *V. uliginosum* and *V. vitis-idaea*, two anthers of each bud were placed between two glass slides that were gently pressed together until pollen grains were released; the slides were then observed at ×60 and the number of pollen tetrads from each anther was counted. For *V. myrtillus* with its numerous pollen grains, individual anthers were excised, squashed in 500 μL lactate-glycerin solution, and vigorously shaken for 5 min. Two samples of 50 μL were taken, deposited on a microscope slide, and examined. The average number of pollen tetrads per anther of each species was multiplied by four and by the number of stamens per flower to estimate the number of pollen grains per flower. The pistils of these 12 buds of each of the three species were dissected under a stereomicroscope and the number of ovules were counted. From these data, pollen to ovule ratios (P:O) were calculated.

Pollen viability was measured by macerating separately two anthers from 10 different flowers of *Vaccinium* (two flowers per individual, one in midbloom, the second in late bloom) in a drop of cotton blue in lactophenol on a microscope slide. Pollen was considered viable and potentially fertile if it appeared normal in shape and the cytoplasm stained dark blue (B. Longly, personal communication). The pollen grains are dispersed as tetrads. Two separate methods were employed to calculate viability. In the first method, only those tetrads that had four viable pollen grains were considered to be viable pollen units and the percentage of viable pollen units was calculated accordingly. In the second method, each pollen grain of a tetrad was scored separately and the percentage of viable pollen grains was calculated, irrespective of the viability of the other grains in the tetrad.

Pollen size measurements (tetrad diameters) were performed by light microscopy with the same slides as those used for pollen viability (50 tetrads per slide).

Stigma receptivity was analyzed in 1988 by observation of pollen tube callose tissue resulting from cross-pollinations at different stages of flower phenology (five flowers of each species examined at anthesis, 1, 2, and 3 days after anthesis).

Other floral traits (corolla diameter, corolla length, style length, anther length and width, anther tube and awn length, and filament length) were measured on three newly opened flowers of four plants of each species, using calipers to 0.1 mm.

Pollination trials

To examine the reproductive biology of the three species, similar-sized branches on 45 patches of each species were marked and all flowers on each branch allocated to one of nine pollination treatments (Table 1). Four treatments were carried out in the presence of pollinators (with effect tested): T1, free exposure (control or open pollination); T2, supplementary cross-pollination to test whether pollen transfer is limited; T3, emasculation, cross-pollination (effect of emasculation); T4, emasculation, no pollination by hand to test whether floral traits provide reproductive assurance. Five treatments involved the use of nylon mesh bags to exclude pollinators: T5, emasculation, cross-pollination that provides the control for T6 and T9 below; T6, emasculation, self-pollination to test for self-compatibility; T7, no emasculation, self-pollination to test whether emasculation has an effect; T8, emasculation, no pollination by hand to test whether agamospermy may occur; T9, no emasculation and no pollination by hand to quantify autonomous self-pollination relative to T5.

All nine treatments were performed in 1990; treatments 1, 2, 4, 5, 6, 7, and 9 were carried out in 1989 and treatments 1, 5, 7, and 9 were carried out in 1988 (Table 1). For the treatments with nylon mesh bags, a wire circle was placed inside the bag to provide a

Table 2. Mean values of floral characters measured on three *Vaccinium* species in the Upper Ardennes (Belgium).

Character	*V. myrtillus*	*V. uliginosum*	*V. vitis-idaea*	*F*	*p*	df
Corolla diameter	5.6±0.2*a*	3.8±0.3*c*	4.9±0.3*b*	171.04	0.0001	37
Corolla length	4.7±0.2*a*	4.8±0.2*a*	5.0±0.2*a*	2.72	0.08	37
Style length	4.7±0.6*b*	3.8±0.2*c*	6.9±0.3*a*	134.8	0.0001	31
Stigma−anther distance	1.9±0.4*b*	1.3±0.2*c*	3.1±0.2*a*	93.8	0.0001	29
Ovules per flower	90.8±15.5*a*	84.9±6.5*a*	64.1±5.5*b*	20.9	0.0001	37
Tetrads per anther	1291.7±290.2*a*	555.0±86.6*b*	1462.3±227.3*a*	68.5	0.0001	32
Anther length	2.3±0.2*a*	0.7±0.1*c*	1.5±0.1*b*	484.2	0.0001	31
Anther width	0.6±0.1*a*	0.5±0.1*b*	0.6±0.1*a*	8.1	0.002	29
Filament length	1.2±0.1*b*	1.1±0.2*b*	1.5±0.1*a*	27.6	0.0001	29
Anther tube length	0.8±0.1*b*	0.7±0.1*c*	1.4±0.1*a*	296.3	0.0001	29
Awn length	0.9±0.1*b*	1.01±0.1*a*	0	19.7	0.0003	20
Tetrad diameter (μm)	46.6±3.7*b*	48.3±2.3*a*	45.1±4.2*b*	7.90	0.002	29
P:O ratio*	582.5±48.4*b*	286.6±31.1*c*	783.8±80.5*a*	9.953	0.0055	61
Pollen viability						
Tetrads (%)	98.4±1.6*a*	92.3±2.7*b*	90.2±6.8*b*	10.732	0.0004	29
Grains (%)	97.8±2.9*a*	97.2±1.2*a*	96.7±2.5*a*	0.575	0.5696	29

Note: Groups that showed significant differences ($p < 0.05$) are indicated with different letters. Unless stated, values are means of 12 measurements and are in millimetres unless stated otherwise.

*Pollen to ovule ratio. Data was log transformed.

frame to keep the netting from collapsing and interfering with floral development. Closure was assured by a hem placed in the underside of the bag. Inflorescences were bagged before flowers opened and all bags were removed at the start of the fruiting season. All the flowers of an inflorescence were pollinated in 1988 and 1989 and 15 flowers per ramet were pollinated in 1990. Flowers were examined daily and hand-pollinated at least twice. Because of the extensive clone size of the species under study, cross-pollinations were carried out using five pollen parents situated at least 100 m (*V. uliginosum* and *V. myrtillus*) or 10 m (*V. vitis-idaea*) from the recipient.

After the flowering period, although the fruits were left to ripen, fruit counts were made weekly to detect any losses due to dissemination and (or) abortion. Mature fruits on ramets in each treatment were harvested in July and August. Fresh fruit weight and diameter were measured, and the number of seeds per fruit counted. In 1988 and 1989, all ovules were counted and categorized as (*i*) mature and fully developed, (*ii*) mostly enlarged and partly filled, or (*iii*) undeveloped. Seed set was thus limited to the first category, whereas fertilized ovules could be in either of the first two categories.

Data analysis

Floral traits, fruit set, and fruit characteristics were compared statistically using one-way analysis of variance (PROC GLM) using SAS (SAS Institute Inc. 1989) and Macintosh STAT VIEW. Percentage of fruit initiated and matured were arcsine transformed prior to being analyzed. In the text, average values are presented with standard deviation (± SD). Means comparisons were also carried out, for floral morphology, using a Scheffé multiple range test. Comparisons among specific treatments were performed by contrast statement following ANOVA in PROC GLM. The contrasts we made are the following: (*i*) contrast 1, pollen transfer limitation: T1 versus T2; (*ii*) contrast 2, autofertility: T9 versus T5; (*iii*) contrast 3, self-compatibility: T6 versus T5; (*iv*) contrast 4, effect of emasculation: T6 versus T7; (*v*) contrast 5, effect of caging: T3 versus T5.

Self-compatibility and autofertility indices

Indices of self-compatibility and autofertility were calculated following Lloyd and Schoen (1992). Self-compatibility is defined as the capacity of a plant to produce zygotes following self-pollination relative to that on outcrossing. Two measures of self-compatibility (SCI) were calculated based on the percentage of fruit and seed set: (*i*) SCI_f = fruit set on emasculated selfing (T6)/fruit set on outcrossing (T5); and (*ii*) SCI_s = seed set on emasculated selfing (T6)/seed set on outcrossing (T5).

A value of one is interpreted as complete self-compatibility, and a value less than 0.75 is interpreted as being due to at least partial self-incompatibility (Lloyd and Schoen 1992). Both seed and fruit set were used because they reflect self-incompatibility and inbreeding depression effects at different levels in the hierarchy of reproductive output.

Autofertility (AFI) was calculated as percentage fruit set (AFI_f) or seed per fruit (AFI_s) on autonomous self-pollination relative to that on outcrossing and thus measures the ability of flowers to self in the absence of pollinators. Two measures of autofertility were estimated: (*i*) AFI_f = fruit set on autonomous self-pollinated (treatment 9)/fruit set on hand outcrossed (treatment 5); and (*ii*) AFI_s = seed per fruit on autonomous self-pollinated (treatment 9)/seed set on hand outcrossed (treatment 5).

To determine whether emasculation reduced fruit and (or) seed set, the effect of emasculation (EE) was determined as EE = fruit or seed set in the emasculated self-pollinated treatment (T6)/fruit or seed set from self-pollinated unemasculated treatment (T7).

Finally, the selfing rate (s) was calculated for each species using the formula of Charlesworth (1988) as s = (MRS in hand-outcrossed (T5) − MRS in free pollinated (T1))/(MRS in hand-outcrossed (T5) − MRS in hand-selfed (T6)), where MRS is the mean reproductive success for a given pollination treatment based on mean seed set per fruit.

Results

Floral morphology

Significant differences among species were found for all floral traits (except percentage of viable pollen and corolla length) (Table 2).

The tetraploid *V. uliginosum* shows several important differences in floral characters relative to the two diploid species. This species has a significantly narrower corolla. *Vaccinium uliginosum* flowers have the longest anther append-

Table 3. ANOVA results for natural fruit set, number of seeds per fruit, and fruit weight, for the three species (A), for the 3 years of experiment (B), and the interaction species × years (AB).

	V. myrtillus		*V. uliginosum*		*V. vitis-idaea*			
Year	Mean ± SD	*N*	Mean ± SD	*N*	Mean ± SD	*N*	*F*(A)	*p*(A)
			Natural fruit set					
1988	46.3±31.1	5	37.7±8.5	5	34.0±16.7	5	0.505	0.6151
1989	20.9±5.8	5	71.6±8.3	5	53.4±25.3	5	13.337	0.0009
1990	48.0±14.4	5	44.0±16.1	5	—		0.177	0.1707
F(B)	2.878		12.216		2.307		A = 1.916	0.1633
p(B)	0.0953		0.0013		0.1631		B = 8.796	0.0009
							AB = 1.744	0.1640
			No. of seeds per fruit					
1988	10.2±8.3	146	23.0±11.1	58	8.7±5.5	39	50.961	0.0001
1989	9.4±9.1	36	11.5±11.2	92	6.8±5.8	66	4.905	0.0084
1990	12.3±9.0	29	16.2±10.2	33	—		2.477	0.1208
F(B)	1.043		19.696		2.959		A = 33.528	0.0001
p(B)	0.3543		0.0001		0.0884		B = 24.001	0.0001
							AB = 7.966	0.0001
			Fruit weight					
1988	171.5±77.5	146	278.7±204.6	35	111.8±48.3	38	23.944	0.0001
1989	171.9±85.4	36	132.0±80.8	93	77.1±43.4	68	23.272	0.0001
1990	193.8±100.4	30	300.0±140.3	32	—		11.350	0.0013
F(B)	1.004		28.795		13.929		A = 23.784	0.0010
p(B)	0.3682		0.0001		0.0003		B = 31.143	0.0001
							AB = 5.665	0.0008

ages (awns), whilst the other floral traits are smaller than in the diploid species (see data for style length, anther width and length, anther tube length, stigma–anther separation, and tetrad number in Table 2). The total pollen grain number is also less in *V. uliginosum* (a mean of 4762 tetrads/flower). In combination, several traits (P:O, stigma–anther separation, corolla width, and anther size) are indicative that this species is likely to self to a greater extent than the two diploid species.

For several of the floral traits, the two diploid species also differ significantly from each other. The corolla of *V. myrtillus* is the more urceolate and larger (5.3–6.0 mm). The corolla and androecium are always pentameric in *V. myrtillus*, and the anthers are the longest of the three species. Its pollen presents the greatest viability based on tetrad units. For *V. myrtillus*, style length, stigma–anther separation, P:O ratio, and anther tube length are intermediate between the two other species (Table 2). This species produces, per anther, a similar number of pollen tetrads (which are of similar size), compared with *V. vitis-idaea*, in anthers of similar width. For ovule number, *V. myrtillus* resembles *V. uliginosum*. Filament length of *V. myrtillus* is nearly the same as that of *V. uliginosum* and its anther awns are shorter than those of this species.

Vaccinium vitis-idaea flowers are diversely oriented, and their styles are exserted and the longest of the three species (2–2.5 mm). It thus has greater stigma–anther separation (herkogamy) than *V. myrtillus* and *V. uliginosum*. The width of the corolla is intermediate to that of the two other species (4.3–5.2 mm) and pollen tetrad viability is similar to that of *V. uliginosum*. *Vaccinium vitis-idaea* has the longest filaments and anther tubes. It produces fewer ovules than the two other species but the same number of pollen tetrads as *V. myrtillus*, hence the highest P:O ratio. For these two important floral characters (pollen to ovule ratio and stigma–anther separation), *V. vitis-idaea* has values that suggest that it is the more outcrossing of the three species.

According to the fluorescent light microscope observations, the flowers of the three *Vaccinium* species are homogamous (*V. vitis-idaea* and *V. uliginosum*) or weakly protandrous (*V. myrtillus*). Anthers of *V. myrtillus* buds were mature 1 day before anthesis, although few pollen grains were released and no pollen tube growth was observed (Jacquemart 1993*b*).

Breeding system

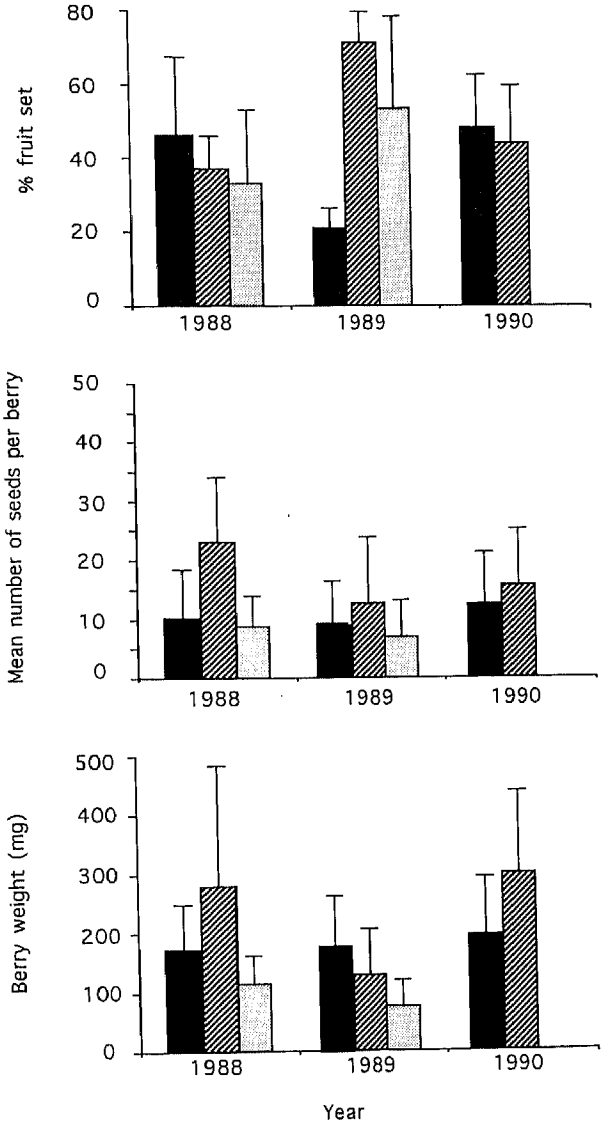
Natural fruit set and general fruit characteristics

Logically, for the three species, mature percent fruit set was highly correlated with percent fruit initiated (in 1989, the mean Pearson correlation coefficients (r) are 0.90 for *V. myrtillus*, 0.97 for *V. uliginosum*, and 0.93 for *V. vitis-idaea*). However, within-treatment fruit set varied markedly (sometimes from 0 to 100%), having a marked effect on our ability to detect statistically significant variation among treatments, ramets, or years.

The number of seeds per fruit was highly correlated with fruit weight (in 1989 $r = 0.85$, 0.68, and 0.77 for *V. myrtillus*, *V. uliginosum*, and *V. vitis-idaea*, respectively). Fruit characteristics (weight and diameter) varied greatly from one berry to another (30, 45, or 62 times for the fruit weight for *V. myrtillus*, *V. uliginosum*, and *V. vitis-idaea*, respectively) and between the different species.

For all three species no evidence for apomixis was found:

Fig. 1. Percentage of natural fruit set, number of seeds per fruit, and fruit weight (T1) for the 3 years in the three *Vaccinium* species at the study site in Ardennes, Belgium. ■, *V. myrtillus*; ▨, *V. uliginosum*; ▩, *V. vitis-idaea*.



no fruits were initiated in T8 (bagged, emasculated, non-pollinated). There was also a significant decrease in fruit set in the emasculated open-pollinated treatment (T4; see Fig. 2).

Differences among species and years

The three species showed marked differences in their pattern of natural fruit set over the 3 years of study (Table 3 and Fig. 1). In 1989, natural fruit set on *V. myrtillus* was significantly lower than the two other species, whereas in 1988 and in 1990, the species showed no significant differences in fruit set (Table 3 and Fig. 1). In 1990, severe frosts during the flowering period of *V. vitis-idaea* caused the death of all its flowers and thus we have no results for this species for this year.

Vaccinium vitis-idaea produced significantly fewer seeds per fruit in all the years of the study (Tables 3 and 4). It also produced significantly lighter fruits than the other two species (Fig. 1 and Table 3). *Vaccinium uliginosum* produced heavier fruits than *V. myrtillus* in 1988 and in 1990 but not in 1989. The mean seed number per fruit was greater for *V. uliginosum* but only significantly in 1988 (Tables 3 and 4).

Table 4. Mean numbers of seeds, fertilized ovules, and undeveloped ovules for the fruits produced in 1989 in the open-pollination treatment for the three *Vaccinium* species (mean ± SD).

Species	Filled seeds	Fertilized ovules	Undeveloped ovules
V. myrtillus	9.4±9.1	44.0±24.3	34.9±23.0
V. uliginosum	11.1±11.6	17.4±10.7	20.1±13.6
V. vitis-idaea	6.8±6.4	22.8±9.6	10.2±6.5

The heaviest fruits containing significantly more seeds occurred in 1988 for *V. vitis-idaea*. The lightest fruits were produced in 1989 for *V. uliginosum* and they contained significantly fewer seeds. Interestingly, changes in fruit set across years were negatively correlated with changes in the mean number of seeds per fruit for *V. uliginosum*. No differences in fruit weight and seed number were detected for *V. myrtillus*.

Differences among pollination treatments

Pollen transfer limitation (contrast T1 vs. T2): In *V. myrtillus*, supplementary cross-pollination (T2) increased fruit set relative to natural fruit set (T1) nonsignificantly in 1989 ($F_{1,6} = 2.477$, $p = 0.166$) and significantly in 1990 ($F_{1,6} = 13.266$; $p = 0.0108$), but supplementary pollination gave significantly higher seed set (1989: $F_{1,75} = 6.687$, $p = 0.0119$; not significantly in 1990: $F_{1,72} = 2.972$, $p = 0.089$); other fruit characteristics did not show significant differences (Table 5).

In *V. uliginosum*, fruit set was greater (but not statistically) after supplementary pollination than in the natural open pollination in both years (Fig. 2; 1989: $F_{1,7} = 0.097$, $p = 0.764$; 1990: $F_{1,7} = 0.119$, $p = 0.7406$). Seed set was statistically greater following supplementary pollination than after natural open pollination in 1989 ($F_{1,147} = 11.649$, $p = 0.0008$) but not in 1990 ($F_{1,27} = 6.027$, $p = 0.0998$). The fruits produced by supplementary pollination were also significantly heavier in 1989 than those produced by the open-pollinated treatment ($F_{1,54} = 4.213$, $p = 0.045$), but the difference was not significant in 1990 ($F_{1,27} = 2.616$, $p = 0.108$).

In *V. vitis-idaea*, there was no significant effect of supplementary pollination on fruit set (Fig. 2; $F_{1,8} = 0.455$, $p = 0.519$) and fruit weight ($F_{1,141} = 2.962$, $p = 0.0875$), although seed set was statistically greater following supplementary pollination ($F_{1,141} = 6.634$, $p = 0.0111$).

Autofertility (T9; T5 vs. T9): The autofertility measures based on fruit set (AFI_f) showed that all the three species could spontaneously set fruits in the absence of pollinators, albeit at low absolute values (see Fig. 2). There was little variation among species for this ability to set fruit in the absence of pollinators (Table 5).

In *V. myrtillus*, the mean percentage of crossed fruit set (T5) was not significantly different in the untreated (T9) fruit set in 1989 ($F_{1,8} = 1.026$, $p = 0.3407$) and significant in

Table 5. Levels of significance of the ANOVA results with the contrasts between treatments, for the three *Vaccinium* species and 2 years, 1989 and 1990 (significance levels: ns, nonsignificant; *, $\alpha < 0.05$; **, $\alpha < 0.01$; ***, $\alpha < 0.001$).

Species	Year	Fruit set	Mean seed no. per fruit	Fruit wt.
Contrast 1 (T1 vs. T2)				
V. myrtillus	1989	ns	*	ns
V. myrtillus	1990	*	ns	ns
V. uliginosum	1989	ns	**	*
V. uliginosum	1990	ns	ns	ns
V. vitis-idaea	1989	ns	*	ns
Contrast 2 (T5 vs. T9)				
V. myrtillus	1989	ns	***	***
V. myrtillus	1990	*	*	**
V. uliginosum	1989	**	ns	ns
V. uliginosum	1990	*	ns	ns
V. vitis-idaea	1989	**	**	**
Contrast 3 (T5 vs. T6)				
V. myrtillus	1989	ns	***	*
V. myrtillus	1990	ns	ns	ns
V. uliginosum	1989	ns	*	*
V. uliginosum	1990	ns	*	*
V. vitis-idaea	1989	ns	**	ns
Contrast 4 (T6 vs. T7)				
V. myrtillus	1989	ns	**	**
V. myrtillus	1990	ns	ns	ns
V. uliginosum	1989	ns	ns	ns
V. uliginosum	1990	*	ns	ns
V. vitis-idaea	1989	ns	ns	**
Contrast 5 (T3 vs. T5)				
V. myrtillus	1990	ns	ns	ns
V. uliginosum	1990	ns	ns	ns

Note: Contrasts were as follows: contrast 1, pollen transfer limitation; contrast 2, autofertility; contrast 3, self-compatibility; contrast 4, emasculation effect; contrast 5, caging effect.

1990 ($F_{1,8} = 7.513$, $p = 0.0254$). In both years, seed set per fruit (and fruit weight) were significantly lower in the spontaneously selfed flowers in comparison with cross-pollinated flowers (1989: $F_{1,51} = 26.666$, $p = 0.0001$; 1990: $F_{1,27} = 7.162$, $p = 0.0125$).

Fruit set was also lower in the untreated spontaneously selfed flowers (T9) compared with hand-selfed flowers (T7) (not significantly in 1989: $F_{1,8} = 5.356$, $p = 0.0694$; significantly in 1990: $F_{1,8} = 11.986$, $p = 0.0085$). Seed set per fruit was not significantly lower in the untreated spontaneously selfed flowers (T9) compared with hand-selfed flowers (T7) (1989: $F_{1,77} = 3.650$, $p = 0.059$; 1990: $F_{1,51} = 3.768$, $p = 0.0506$). However, the differences in fruit weight were significant (1989: $F_{1,8} = 10.726$, $p = 0.0016$; 1990: $F_{1,51} = 7.306$, $p = 0.0093$).

The values calculated s were 1.074 in 1989 and 0.483 in 1990.

In *V. uliginosum*, the trends are opposite to those presented by *V. myrtillus*. The mean percentage of hand-crossed fruit set was significantly higher than untreated fruit set (1989: $F_{1,8} = 26.051$, $p = 0.0022$; 1990: $F_{1,8} = 10.158$, $p = 0.0153$), but seed set per fruit was not increased significantly (1989: $F_{1,40} = 0.440$, $p = 0.5109$; 1990: $F_{1,18} = 0.018$, $p = 0.8944$), as well as fruit weight (Table 5).

Seed set per fruit did not decrease in the untreated spontaneously selfed flowers (T9) compared with hand-selfed flowers (T7) (Fig. 3; 1989: $F_{1,37} = 0.106$, $p = 0.7465$; 1990: $F_{1,18} = 0.005$, $p = 0.9455$) as well as fruit weight (Table 5). Fruit set was lower in the untreated spontaneously selfed flowers (T9) compared with hand-selfed flowers (T7) (not significantly in 1989: $F_{1,6} = 2.518$, $p = 0.1636$; significantly in 1990: $F_{1,5} = 10.620$, $p = 0.0225$).

The values of s were negative (−6.555 in 1989 and −3.091 in 1990), indicating better results with open-pollinated flowers (deleterious emasculation effect; see above).

In *V. vitis-idaea*, the mean percentage of hand-crossed fruit set was significantly higher than untreated fruit set

Fig. 2. Percentage of fruit set for the different pollination treatments applied in 1989 to *V. myrtillus*, *V. uliginosum*, and *V. vitis-idaea*. See Table 1 for treatment codes. *N*, number of samples.

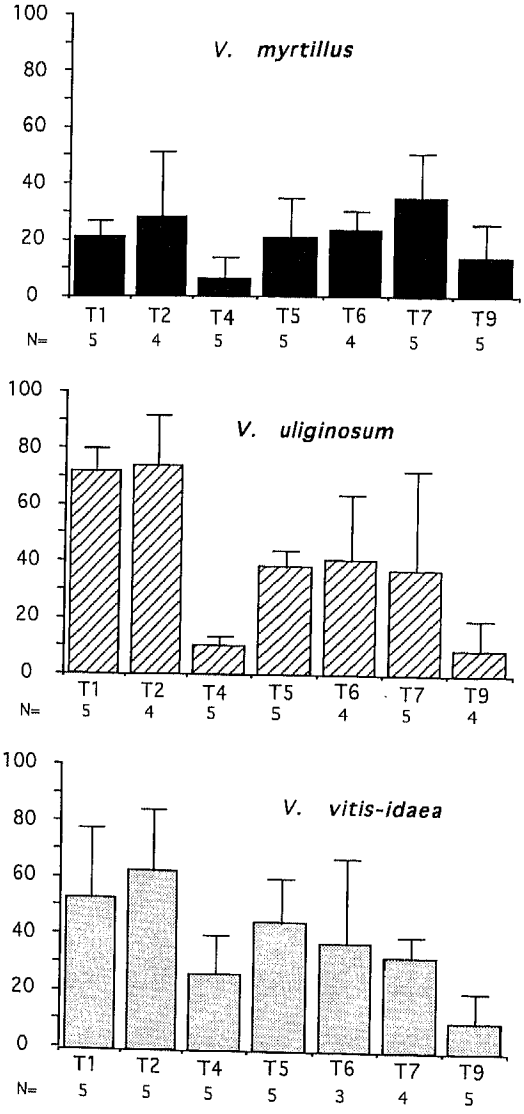
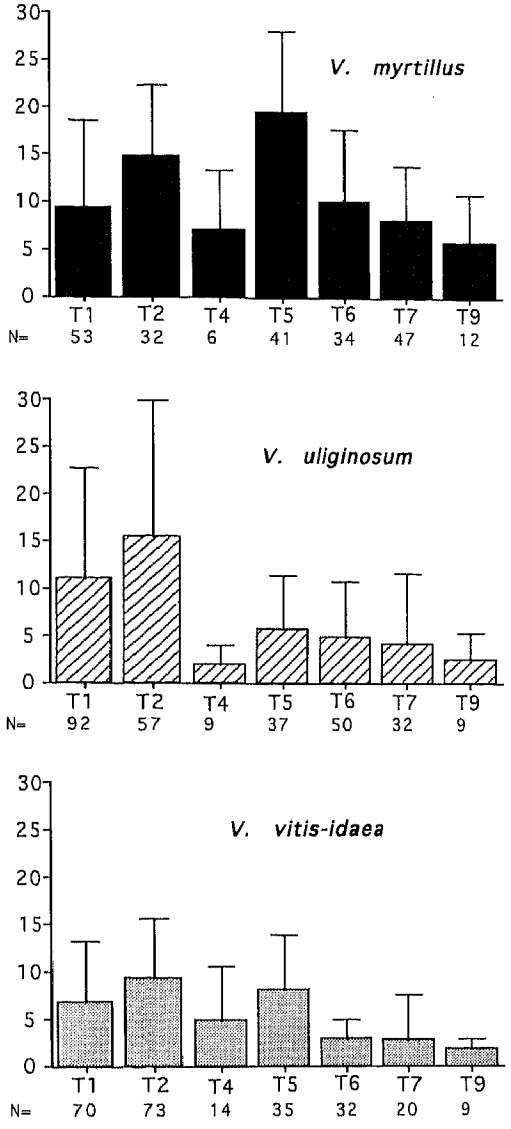


Fig. 3. Mean seed number per fruit for the different pollination treatments applied in 1989 to *V. myrtillus*, *V. uliginosum*, and *V. vitis-idaea*. See Table 1 for treatment codes. *N*, number of samples.



($F_{1,8}$ = 15.143, p = 0.0046). Fruit set was also lower in the untreated spontaneously selfed flowers (T9) compared with hand-selfed flowers (T7) ($F_{1,7}$ = 8.869, p = 0.0206).

Seed set per fruit was significantly lower in the spontaneously selfed flowers compared with hand cross-pollinated flowers ($F_{1,42}$ = 10.320, p = 0.0025) as well as fruit weight. However, seed set per fruit (and fruit weight) did not decrease in the untreated spontaneously selfed flowers (T9) compared with hand-selfed flowers (T7) (Fig. 3; $F_{1,22}$ = 1.503, p = 0.2331). The value of s was 0.250.

Self-compatibility (T5 vs. T6): The indices of self-compatibility (SCI; Table 6) based on fruit set (SCI_f) of the three *Vaccinium* species were highly variable among years and species, ranging from 0.84 in *V. vitis-idaea* to 1.06 in *V. uliginosum* and 1.12 in *V. myrtillus* (Table 6, in 1989). Those obtained for seed number (SCI_s) were 0.36 in *V. vitis-idaea*, 0.52 for *V. myrtillus*, and 0.84 for *V. uliginosum* (in 1989). The indices obtained with seed set were 0.58 for *V. vitis-idaea*, 0.56 for *V. uliginosum*, and 0.38 for *V. myrtillus*. Although slightly variable, those results suggest a lower level of self-compatibility in *V. vitis-idaea* than in the two other species. As a result, the SCI_s for *V. uliginosum* was greater than those of *V. myrtillus* and *V. vitis-idaea*.

Table 6. Self-compatibility (SCI) and autofertility (AFI) indices for the three *Vaccinium* species based on percent fruit set (AFI_f and SCI_f), percent seed set (AFI_s and SCI_s), and the mean number of seeds per fruit, in 1989 and 1990 (only in 1989 for *V. vitis-idaea*).

	Fruit set		% seed set	Seeds per fruit	
	1989	1990	(1989)	1989	1990
V. myrtillus					
AFI	0.66	0.35	0.26	0.32	0.25
SCI	1.12	1.11	0.38	0.52	0.56
V. uliginosum					
AFI	0.18	0.23	0.64	0.43	0.30
SCI	1.06	0.47	0.59	0.84	0.41
V. vitis-idaea					
AFI	0.23	—	0.29	0.22	—
SCI	0.84	—	0.58	0.36	—

Note: AFI is fruit and seed set on autonomous selfing relative to hand outcrossing, and SCI is fruit and seed set on hand selfing relative to hand outcrossing.

The three species showed slightly better performance with fruit characteristics following cross-pollination than following self-pollination (T5, T6, and T7). Based on fruit and seed sets, all species were highly self-compatible (Table 6). The three species did not show any significant difference in fruit set (T6 vs. T7), but they showed a significant difference between selfed and outcrossed seed per fruit (only not significant in 1990 for *V. myrtillus*). However, a low fruit set in emasculated crossed (T5) and emasculated selfed (T6) treatments was often observed, which may be a consequence of emasculation since it was relatively difficult to emasculate the flowers without damage, especially for the dense racemes of *V. vitis-idaea*. This, however, is extremely variable.

For *V. myrtillus*, in 1989, the comparison of T5 (crossing) versus T6 (selfing) gave the following results: the fruits produced by crossing were heavier than those produced by selfing ($F_{1,79}$ = 19.171, p = 0.0001) and the results obtained with seed set per fruit were significantly higher ($F_{1,79}$ = 38.479, p = 0.0001). However, the differences in fruit set were not significant ($F_{1,7}$ = 0.204, p = 0.6653). In 1990, no significant differences were detected ($F_{1,8}$ = 0.039, p = 0.8474 for fruit set; $F_{1,42}$ = 1.081, p = 0.3044 for seed number). Moreover, the differences were significant with mean number of partly filled seeds (31.4 ± 22.0 per fruit for T5 and 60.6 ± 25.8 per fruit for T6; $F_{1,79}$ = 29.982, p = 0.0001) and with mean number of undeveloped ovules per fruit (16.6 ± 8.9 for T5 and 28.0 ± 20.4 for T6; $F_{1,79}$ = 9.413, p = 0.003).

For *V. uliginosum*, in both years, the comparison of T5 (crossing) versus T6 (selfing) gave the following results: the fruits produced by crossing were heavier than those produced by selfing (1989: $F_{1,83}$ = 6.080, p = 0.0157; 1990:

$F_{1,20} = 6.120$, $p = 0.0224$) and contained more seeds (1989: $F_{1,83} = 6.764$, $p = 0.0110$; 1990: $F_{1,20} = 6.603$, $p = 0.0183$). However, selfed fruits did not differ significantly in the number of partly filled seeds per fruit (19.1 ± 16.2 for T5 and 16.0 ± 12.0 for T6; $F_{1,34} = 1.024$, $p = 0.3145$) and the number of ovules per fruit (23.3 ± 16.3 for T5 and 30.4 ± 2.7 for T6; $F_{1,34} = 3.231$, $p = 0.0759$) compared with crossed fruits. The differences in fruit set were not significant (1989: $F_{1,7} = 0.035$, $p = 0.8573$; 1990: $F_{1,7} = 5.397$, $p = 0.0532$).

For *V. vitis-idaea*, in 1989, the comparison of T5 versus T6 gave the following results: the fruits produced by crossing were not heavier than those produced by selfing ($F_{1,50} = 0.999$, $p = 0.3223$), but they contained significantly more seeds ($F_{1,50} = 10.887$, $p = 0.0018$), more partly filled seeds (18.9 ± 8.6 vs. 12.7 ± 6.2; $F_{1,63} = 10.567$, $p = 0.0019$) and similar number of ovules per fruit (7.2 ± 4.8 vs. 8.0 ± 4.1; $F_{1,63} = 0.457$, $p = 0.5015$). The differences in fruit set were not significant ($F_{1,6} = 0.251$, $p = 0.6342$).

Effects of emasculation and caging (T6 vs. T7 and T3 vs. T5): In *V. myrtillus*, a significant deleterious effect of emasculation (EE; seed set and other fruit characteristics in T6 relative to that in T7) was found in 1989 (Fig. 2; for seed number: $F_{1,79} = 8.834$, $p = 0.0037$; for fruit weight: $F_{1,100} = 8.656$, $p = 0.004$) but not in 1990 ($F_{1,66} = 0.378$, $p = 0.5408$ for seed number and $F_{1,284} = 1.284$, $p = 0.2612$ for fruit weight) and not for fruit set in both years (1989: $F_{1,7} = 1.702$, $p = 0.2333$; 1990: $F_{1,8} = 2.283$, $p = 0.1692$).

The effect of caging was measured by comparing the bagged hand-outcrossed treatment (T5) with the open hand-outcrossed treatment (T3) in 1990. This effect was not significant (for fruit set: $F_{1,7} = 1.833$, $p = 0.2178$; for seed set: $F_{1,47} = 0.421$, $p = 0.5198$; for fruit weight: $F_{1,47} = 0.196$, $p = 0.6601$).

In *V. uliginosum*, emasculation had a significant deleterious effect only on fruit diameter in 1989 ($F_{1,80} = 3.202$, $p = 0.0774$) and on fruit set in 1990 ($F_{1,5} = 8.249$, $p = 0.0349$).

The effect of caging was measured by comparing the bagged hand-outcrossed treatment (T5) with the open hand-outcrossed treatment (T3) in 1990. This effect was not significant (for seed set: $F_{1,53} = 3.731$, $p = 0.0588$; for fruit set: $F_{1,7} = 0.0035$, $p = 0.9855$; for fruit weight: $F_{1,37} = 2.992$, $p = 0.092$).

In *V. vitis-idaea*, emasculation had a significant deleterious effect on fruit weight ($F_{1,50} = 7.414$, $p = 0.0089$) and diameter ($F_{1,50} = 23.738$, $p = 0.0774$) but not on fruit ($F_{1,6} = 0.251$, $p = 0.6342$) and seed ($F_{1,42} = 0.690$, $p = 0.4109$) sets.

The lack of results in 1990 for *V. vitis-idaea* precludes the comparison of the bagged hand-outcrossed treatment (T5) with the open hand-outcrossed treatment (T3) to estimate the caging effect.

Discussion

Variation in floral traits among the species

Our study of the floral biology and pollination ecology of three sympatric *Vaccinium* species shows that all the three species are highly self-compatible, although floral traits and levels of autofertility and self-compatibility suggest that the realized level of selfing versus outcrossing in natural populations is likely to vary among years and among species.

The flowers of the three *Vaccinium* species are homogamous (*V. vitis-idaea* and *V. uliginosum*) or weakly protandrous (*V. myrtillus*). These observations are in agreement with those of Bertin and Newman (1993), who stated that the majority of ericaceous species (39 out of 64 examined) are protandrous (only 8 out of 64 were homoganous). Protandry is not a particularly effective mechanism (relative to protogyny for example) to reduce self-pollination (Bertin 1993), although it is correlated with growth form and life-history traits such as the angiosperm habit, shrubby form, and bee pollination (Bertin and Newman 1993).

Clear-cut differences between the species occurred in floral traits, particularly those related to the mating system, namely stigma–anther separation, corolla width, style length, pollen to ovule ratio, and all anther dimensions. For these traits, the polyploid species *V. uliginosum* has characters that indicate that this species has a more selfing mating system than the two diploid species. Indeed this species showed greater autofertility and self-compatibility than the two diploids. To what extent this is related to the genetic consequences associated with polyploidy is particularly interesting (see Thompson and Lumaret 1992).

Vaccinium vitis-idaea had values that suggest a floral phenotype more adapted to cross-pollination than the two other species. Of particular importance here is the increased stigma–anther separation that has often been found to markedly decrease autonomous selfing (Barrett 1989; Holtsford and Ellstrand 1992; Ramsey 1993; L. Affre and J.D. Thompson, unpublished data). *Vaccinium vitis-idaea* flowers are diversely oriented and their anthers are awnless (Table 2). This suggests that the pollen release strategy is different, i.e., buzz pollination (Buchmann 1983; Knudsen and Olesen 1993) does not occur (and the flowers are visited by honeybees that cannot buzz; Harder and Barclay 1994) and also that there is less spontaneous selfing because of the pendulous nature of the flowers as in the two other species (anemo-autogamy according to Hagerup 1928). This species also has the largest total number of flowers per inflorescence. Its daily inflorescence size (several flowers simultaneously open) is also greater and means that honeybees, the major pollinators in our study population, can visit many flowers. Although floral traits suggest that this species is more outcrossing, it may suffer more geitonogamy because of its inflorescence biology.

Stigma–anther separation is correlated with increased stigma exertion in *V. vitis-idaea* and fits with a generally accepted cross-pollination syndrome (Moore and Lewis 1964; Schoen 1982*b*; Robertson and Lloyd 1991). In this context, *V. myrtillus* and *V. uliginosum* have a floral morphology and P:O ratio suggesting greater values of self-fertilization. These findings are consistent with predictions of reduced male function and increased female function in self-fertilizing plants (Cruden 1977; Charlesworth and Charlesworth 1987). It is of interest that *V. vitis-idaea* flowers also have both the greatest number of pollen grains and the lowest number of ovules.

Reduced pollen production and P:O ratios in selfing plants

were reported frequently in both interspecific and intraspecific comparisons (Cruden 1977; Wyatt 1984). Selfing plants can also increase female function, without altering the number of ovules per flower, by increasing the number of flowers that produce fruit (Lloyd 1987). Increased seed production was indeed observed in *V. uliginosum*, which is considered here to be the most highly self-pollinated species of the three. In *V. vitis-idaea*, seed set was almost half that of *V. uliginosum*. Of particular interest here is the fact that the difference between the number of fertilized ovules and filled seeds was markedly less in the supposed selfer (*V. uliginosum*) than in the two supposed more outcrossing species (Table 4). Elsewhere, embryonic genetic load was involved to interpret such ovule abortion in outcrossing species (Wiens et al. 1987). Indeed in other outcrossing *Vaccinium* species, abortion of selfed ovules was found to exceed that of outcrossed ovules, indicative that early inbreeding depression may limit selfing (Krebs and Hancock 1991). The fact that *V. uliginosum* shows much reduced selfed embryo abortion may be a further indication of its selfing nature.

Fruit set, seed set, and pollen limitation

The three species showed marked differences in their pattern of natural fruit set over the 3 years of the study (Fig. 1). In 1989, natural fruit set on *V. myrtillus* was lower than the two other species. In 1988, however, *V. myrtillus* showed greater natural fruit set than the two other species. The differences in flowering periods for the three *Vaccinium* species could explain these facts (Jacquemart 1993*b*). In 1988 and 1990, the weather was warm and sunny during the flowering period of *V. myrtillus*. After this favorable period, rains and frosts in June, during the flowering periods of the other species, are the probable cause of reduced fruit set. In 1989, however, a cold period when *V. myrtillus* flowered is thought to be the cause of its lower fruit set. In 1990, severe frosts during the flowering period of *V. vitis-idaea* caused the death of all its flowers; we have no results for this species for this year.

Values of natural fruit set are similar to those of other fleshy-fruited species (Coombe 1976) and other *Vaccinium* species (Ritchie 1955*a*; Free 1970; Lehmushovi 1977; Cane et al. 1985). Between-year variation in fruit set in *V. myrtillus* and *V. vitis-idaea* was frequently reported (Lehmushovi 1977; Belonogova 1988; Kuchko 1988), illustrating the importance of studies over several years. The explanations proposed to account for such variation include a range of factors associated with climatic variation: (*i*) late spring frosts (Lehmushovi 1977; Belonogova 1988), (*ii*) heat and moisture variation during flower bud formation (Kuchko 1988), and (*iii*) decreased pollinator visits during inclement weather periods (Lehmushovi 1977; Kuchko 1988). Floral herbivory was also found to be important (Flowers-Ellis 1971; Kuchko 1988).

In our study, such variation may also have been introduced by the manipulations we performed, particularly emasculation of flower buds (T2; not really a reproductive assurance), which may remove signals and (or) rewards for pollinators (Schoen and Lloyd 1992). Such manipulations involved removal of a great part of the corolla as well as pollen, which may have reduced pollinator visitation and male function. Supplementary pollination increased fruit set and produced heavier fruits that regularly contained more seeds than those in open pollination; some of these differences were significant, suggesting pollen transfer limitation (for example, for *V. uliginosum*, in 1990 and for all fruit characteristics).

Autofertility and auto-compatibility indices

The indices of self-compatibility reviewed by Lloyd and Schoen (1992) for 66 species range continuously from 0 to more than 1. Thus, there is a gradient from partially self-incompatible to self-compatible plants. Self-compatibility can thus be considered as a quantitative phenomenon (as further argued by Becerra and Lloyd 1992). Samples with indices greater than 0.75 are described by Lloyd and Schoen (1992) as self-compatible species. The three species would thus be considered as self-compatible based on fruit set values; however, given that seed set is the ultimate value with which to quantify self-compatibility, it would appear that the three species show at least partial self-incompatibility or early-acting inbreeding depression (Table 6 and see above).

The autofertility index of Lloyd and Schoen (1992) also ranged continuously from 0 to greater than 1 in the species reviewed, indicating that all degrees of autonomous self-pollination may occur. Our values ranged from 0.18 to 0.66 for fruit set in 1989 and from 0.22 to 0.43 for seed set (Table 6). A value of 1 for the autofertility index indicates that there are no barriers to autonomous self-pollination, and low values indicate that autonomous self-pollination is rare (Lloyd and Schoen 1992; Kron et al. 1993). Thus our data suggest that the species are highly variable in their autonomous selfing capacity. As predicted, *V. vitis-idaea* shows the lowest autofertility index of the three species.

Although the self-compatibility estimates were also highly variable for the three species (Table 6), the results fit our previous data on floral biology. The estimate of the selfing rate of *V. myrtillus* obtained in 1990 using mean seed number per fruit is similar to the values of 0.25–0.34 obtained with isozymes (Jacquemart et al. 1994). Unfortunately, we have no estimation with isozymes for the two other species. For *V. vitis-idaea*, the selfing rate was lower than that of the two other species, as was its autonomous selfing capacity (see above). The deleterious effect of emasculation does not permit estimation of the selfing rate for *V. uliginosum* (more fruits and higher fruit characteristics in T1 than in T5).

According to Free (1970) and Reader (1977), ericaceous species are in general self-compatible. Nevertheless, the majority of the American *Vaccinium* species, including *V. angustifolium* Ait., *V. myrtilloides* Michx., *V. macrocarpon* Ait. (Reader 1977; Mohr and Kevan 1987; Rabaey and Luby 1988), *V. corymbosum* L., *V. darrowi* Camp., *V. tenellum* Ait., and *V. virgatum* Ait (Knight and Scott 1964; Mohr and Kevan 1987; Rabaey and Luby 1988; Vander Kloet 1991), show a reduction of fruit set after self-fertilization. Harrison et al. (1993) suggested that the term self-incompatibility should not be used because there did not appear to be a prefertilization barrier. However, the self-incompatibility phenomenon can also be late-acting (see Seavey and Bawa 1986) and it is in practice extremely difficult to distinguish this from embryonic inbreeding depression (Krebs and Hancock 1988, 1991; Jones 1994).

Four factors potentially contribute to the selfing rate in any species: (*i*) autonomous within-flower self-pollination; (*ii*) facilitated within-flower self-pollination; (*iii*) geitonog-

amy; and (*iv*) biparental inbreeding. Our autofertility indices indicate that autonomous self-pollination is likely to be rare but possible. Although autonomous self-pollination may occur in *Vaccinium* species, insect-mediated self-pollination likely accounts for the majority of fruits and seeds produced by selfing and therefore contributes more to the selfing rate than autonomous self-pollination. This is supported by the fact that most seed and fruit set in free-pollinated flowers can be accounted for by insect pollination (Jacquemart 1993*a*). For example, in *V. uliginosum*, open-pollinated flowers had on average 56% fertilized ovules compared with 40% for flowers from which pollinators were excluded (but which set fruit). This difference is most apparent for fruit set where 72% fruit set occurred on naturally pollinated and only 9% on autonomous selfing (Fig. 2). The relative importance of autonomous self-pollination to the realized selfing rate will vary with pollinator activity, since autonomous self-pollination will account for a relatively higher proportion of seeds set when pollinator activity is low.

Pollinator-mediated selfing can be either autogamous (facilitated self-pollination) or geitonogamous. In the three *Vaccinium* species both are possible and probably occur, since the SCI values indicate that the three species are at least partly self-compatible. However, the fact that natural pollinators visit many flowers on a single plant (Jacquemart 1993*a*) suggests that geitonogamy is likely to be of great importance to the selfing rate. The main pollinators are bumblebees that are known to cause high levels of within-plant pollen transfer in densely flowered plants (Klinkhamer et al. 1994), which are common in densely flowering, clonal species (Handel 1985; Kron et al. 1993; Klinkhamer et al. 1994), although this depends to some extent on spatial clonal organization (Leclerc-Potvin and Ritland 1994).

In conclusion, our data show that variation in floral traits, autofertility, and self-compatibility among species suggests a more selfing mating system in the polyploid species. However, low fruit set in these species does not occur because of pollen limitation.

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References

Barrett, S.C.H. 1989. Mating system and speciation in heterostylous plants. *In* Speciation and its consequences. *Edited by* D. Otte and J.A. Endler, Sinauer Associates Inc., Sunderland, Mass. pp. 257–283.

Barrett, S.C.H., and Eckert, C.G. 1990. Variation and evolution of mating systems in seed plants. *In* Biological approaches and evolutionary trends in plants. *Edited by* S. Kawano. Academic Press, London. pp. 229–254.

Becerra, J., and Lloyd, D.G. 1992. Competition-dependent abscission of self-pollinated flowers of *Phormium tenax* (Agavaceae): a second action of self-incompatibility at the whole-flower level. Evolution, **46**: 458–469.

Belonogova, T.V. 1988. Yield forecasting and optimization of fruit harvesting in the forests of Southern Karelia, USSR. Acta Bot. Fenn. **136**: 19–21.

Bertin, R.I. 1993. Incidence of monoecy and dichogamy in Angiosperms. Am. J. Bot. **80**: 557–583.

Bertin, R.I., and Newman, C.M. 1993. Dichogamy in Angiosperms. Bot. Rev. **59**: 112–152.

Buchmann, S.L. 1983. Buzz pollination in Angiosperms. *In* Handbook of experimental pollination biology. *Edited by* C.E. Jones and R.J. Little, Van Nostrand, Reinhold, New York. pp. 73–115.

Cane, J.H., Eickwort, G.C., Wesley, F.R., and Spielholz, J. 1985. Pollination ecology of *Vaccinium stamineum* (Ericaceae, Vaccinioidae). Am. J. Bot. **72**: 135–142.

Charlesworth, D. 1988. A method for estimating outcrossing rates in natural populations of plants. Heredity, **61**: 469–471.

Charlesworth, D., and Charlesworth, B. 1987. Inbreeding depression and its evolutionary consequences. Annu. Rev. Syst. Ecol. **18**: 237–268.

Clapham, A.R., Tutin, T.G., and Warburg, E.F. 1962. Flora of the British Isles. 2nd ed. Cambridge University Press, London.

Coombe, B.G. 1976. The development of fleshy fruits. Annu. Rev. Plant Physiol. **27**: 507–528.

Cruden, R.W. 1977. Pollen–ovule ratios: a conservative indicator of breeding systems in flowering plants. Evolution, **1**: 32–46.

Cruden, R.W., and Lyon, D.L. 1985. Patterns of biomass allocation to male and female functions in plants with different mating systems. Oecologia, **66**: 299–306.

Cruden, R.W., and Lyon, D.L. 1989. Facultative xenogamy: examination of a mixed mating system. *In* The evolutionary ecology of plants. *Edited by* J.H. Bock and Y.B. Linhart. Westview Press, Boulder, Colo. pp. 171–207.

Dole, J.A. 1992. Reproductive assurance mechanisms in three taxa of the *Mimulus guttatus* complex (Scrophulariaceae). Am. J. Bot. **79**: 650–659.

Flowers-Ellis, J.G.K. 1971. Age structure and dynamics in stands of bilberry (*Vaccinium myrtillus* L.). Avdel. Skoqsek. Stock. Rapp. Upps. **9**: 1–108.

Free, J.B. 1970. Insect pollination of crops. Academic Press, London.

Gimingham, C.H. 1972. Ecology of heathlands. Chapman & Hill, London.

Hagerup, O. 1928. Morphological and cytological studies of bicorns. Dan. Bot. Ark. **6**: 1–27.

Handel, S.N. 1985. The intrusion of clonal growth patterns on plant breeding systems. Am. Nat. **125**: 367–384.

Harder, L.D., and Barclay, M.R. 1994. The functional significance of poricidal anthers and buzz pollination: controlled pollen removal from *Dodecatheon*. Funct. Ecol. **8**: 509–517.

Harrison, R.E., Luby, J.J., and Ascher, P.D. 1993. Genetic characteristics of self fertility in highbush and half-high blueberries. Euphytica, **67**: 79–88.

Holtsford, T.P., and Ellstrand, N.C. 1992. Genetic and environmental variation in floral traits affecting outcrossing rate in *Clarkia tembloriensis* (Onagraceae). Evolution, **46**: 216–225.

Jacquemart, A.-L. 1992. Préliminaires sur la production de nectar chez trois espèces de *Vaccinium*. Apidologie, **23**: 453–464.

Jacquemart, A.-L. 1993*a*. Floral visitors of *Vaccinium* species in the High Ardennes, Belgium. Flora (Jena), **187**: 67–72.

Jacquemart, A.-L. 1993*b*. Écologie du système reproducteur chez trois espèces de *Vaccinium* en haute Ardenne. Ph.D. thesis, University of Louvain, Louvain-la-Neuve, Belgium.

Jacquemart, A.-L., Mahy, G., Raspé, O., and De Sloover, J.R. 1994. An isozyme study in bilberry (*Vaccinium myrtillus*). 2. Mating system and genetic structure. Belg. J. Bot. **127**: 105–114.

Jones, K.N. 1994. Nonrandom mating in *Clarkia gracilis* (Onagraceae): a case of cryptic self-incompatibility. Am. J. Bot. **81**: 195–198.

Klinkhamer, P.G.L., de Jong, T.J., and Metz, J.A.J. 1994. Why plants can be too attractive—a discussion of measures to estimate male function. J. Ecol. **82**: 191–194.

Knight, S.J., and Scott, D.H. 1964. Effects of temperature on self- and cross-pollination and fruiting of four highbush blueberry varieties. J. Am. Soc. Hortic. Sci. **85**: 302–306.

Knudsen, J.T., and Olesen, M.J. 1993. Buzz-pollination and patterns in sexual traits in north european Pyrolaceae. Am. J. Bot. **80**: 900–913.

Krebs, S.L., and Hancock, J.F. 1988. The consequences of inbreeding on fertility in *Vaccinium corymbosum* L. J. Am. Soc. Hortic. Sci. **113**: 914–918.

Krebs, S.L., and Hancock, J.F. 1991. Embryonic genetic load in the highbush blueberry, *Vaccinium corymbosum* (Ericaceae). Am. J. Bot. **78**: 1427–1437.

Kron, P., Stewart, S.C., and Back, A. 1993. Self-compatibility, autonomous self-pollination, and insect-mediated pollination in the clonal species *Iris versicolor*. Can. J. Bot. **71**: 1503–1509.

Kuchko, A.A. 1988. Bilberry and cranberry yields and the factors controlling them in the forests of Karelia, USSR. Acta Bot. Fenn. **136**: 23–25.

Leclerc-Potvin, C., and Ritland, K. 1994. Modes of self-fertilization in *Mimulus guttatus* (Scrophulariaceae)—a field experiment. Am. J. Bot. **81**: 199–205.

Lehmushovi, A. 1977. Some aspects of the cowberry trials in Finland. Ann. Agric. Fenn. **16**: 57–63.

Lloyd, D.G. 1987. Allocations to pollen, seeds and pollination mechanisms in self-fertilizing plants. Funct. Ecol. **1**: 83–89.

Lloyd, D.G., and Schoen, D.J. 1992. Self-fertilization and cross-fertilization in plants. 1. Functional dimensions. Int. J. Plant Sci. **153**: 358–369.

Lloyd, D.G., and Webb, C.J. 1977. Secondary sex characters in seed plants. Bot. Rev. **43**: 177–211.

Matthews, J.R., and Knox, E.M. 1926. The comparative morphology of the stamen in Ericaceae. Trans. Proc. Bot. Soc. Edinb. **29**: 243–281.

Mazer, S.J., and Hultgard, U.-M. 1993. Variation and covariation among floral traits within and among four species of Northern european *Primula* (Primulaceae). Am. J. Bot. **80**: 474–485.

Mohr, N.A., and Kevan, P.G. 1987. Pollinators and pollination requirements of lowbush blueberry (*Vaccinium angustifolium* Ait. and *V. myrtilloides* Michx.) and cranberry (*V. macrocarpon* Ait.) in Ontario with notes on highbush blueberry (*V. corymbosum* L.) and ligonberry (*V. vitis-idaea* L.). Proc. Entomol. Soc. Ont. **118**: 149–155.

Moore, D.M., and Lewis, H. 1964. The evolution of self-pollination in *Clarkia xantiana*. Evolution, **19**: 104–114.

Pickering, C.M., and Ash, J.E. 1993. Gender variation in hermaphrodite plants: evidence from five species of alpine *Ranunculus*. Oikos, **68**: 539–548.

Rabaey, A., and Luby, J. 1988. Fruit set in half high-blueberry genotypes following self- and cross-pollination. Fruit Var. J. **42**: 126–130.

Ramsey, M. 1993. Floral morphology, biology and sex allocation in disjunct populations of Christmas Bells (*Blandfordia grandiflora*, Liliaceae) with different breeding systems. Aust. J. Bot. **41**: 749–762.

Reader, R.J. 1977. Bog ericad flowers: self-compatibility and relative attractiveness to bees. Can. J. Bot. **55**: 2279–2287.

Ritchie, J.C. 1955*a*. Biological flora of the British Isles: *Vaccinium vitis-idaea*. J. Ecol. **43**: 701–708.

Ritchie, J.C. 1955*b*. A natural hybrid in *Vaccinium*. I. The structure, performance and chorology of the cross *Vaccinium intermedium* Ruthe. New Phytol. **54**: 49–67.

Ritchie, J.C. 1956. Biological flora of the British Isles: *Vaccinium myrtillus*. J. Ecol. **44**: 291–299.

Ritland, C., and Ritland, K. 1989. Variation of sex allocation among eight taxa of the *Mimulus guttatus* species complex (Scrophulariaceae). Am. J. Bot. **76**: 1731–1739.

Robertson, A.W., and Lloyd, D.G. 1991. Herkogamy, dichogamy and self-pollination in 6 species of *Myosotis* (Boraginaceae). Evol. Trends Plants, **5**: 53–63.

Rousi, A. 1967. Cytological observations on some species and hybrids of *Vaccinium*. Zuechter, **36**: 352–359.

SAS Institute Inc. 1989. SAS user's guide, SAS Institute, Cary, N.C.

Schemske, D.W. 1983. Breeding system and habitat effects on fitness components in three neotropical *Costus* (Zingiberaceae). Evolution, **37**: 523–539.

Schoen, D.J. 1982*a*. Male reproductive effort and breeding system in an hermaphroditic plant. Oecologia, **53**: 255–257.

Schoen, D.J. 1982*b*. The breeding system of *Gilia achilleifolia* variation in floral characteristics and outcrossing rate. Evolution, **36**: 352–360.

Schoen, D.J., and Lloyd, D.G. 1992. Self-fertilization and cross-fertilization in plants. 3. Methods for studying modes and functional aspects of self-fertilization. Int. J. Plant Sci. **153**: 381–393.

Seavey, S.R., and Bawa, K.S. 1986. Late-acting self-incompatibility in Angiosperms. Bot. Rev. **52**: 195–219.

Stewart, W.S., and Bannister, P. 1973. Seasonal changes in carbohydrate content of three *Vaccinium* species with particular reference to *Vaccinium uliginosum* L. and its distribution in the British Isles. Flora (Jena), **162**: 134–155.

Thompson, J.D., and Lumaret, R. 1992. The evolutionary dynamics of polyploid plants: origins, establishment and persistence. TREE, **7**: 302–307.

Uotila, P., and Pellinen, K. 1985. Chromosome numbers in vascular plants from Finland. Acta Bot. Fenn. **130**: 7.

Uyenoyama, M.K. 1986. Inbreeding and the cost of meiosis: the evolution of selfing in populations practicing biparental inbreeding. Evolution, **40**: 388–404.

Vander Kloet, S.P. 1991. The consequences of mixed pollination on seed set in *Vaccinium corymbosum* L. Can. J. Bot. **69**: 2448–2454.

Warming, E. 1908. The structure and biology of Arctic flowering plants. I. Ericineae. I. Morphology and biology. Medd. Gronl. **36**: 1–71.

Wiens, D., Calvin, C.L., Wilson, C.A., Davern, C.I., Frank, D., and Seavey, S.R. 1987. Reproductive success, spontaneous embryo abortion, and genetic load in flowering plants. Oecologia, **71**: 501–509.

Wyatt, R. 1984. The evolution of self-pollination in granite outcrop species of *Arenaria* (Caryophyllaceae). III. Reproductive effort and pollen–ovule ratios. Syst. Bot. **9**: 432–440.

Wyatt, R. 1988. Phylogenetic aspects of the evolution of self-pollination. *In* Plant evolutionary biology. *Edited by* L.D. Gottlieb and S.K. Jain. Chapman and Hall, London. pp. 109–131.