

FLORAL TRAITS OF BELGIAN ERICACEAE SPECIES : ARE THEY GOOD INDICATORS TO ASSESS THE BREEDING SYSTEMS ?

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ABSTRACT. — A variety of floral traits likely to be associated with the reproductive system of seven Belgian Ericaceae species (four *Vaccinium* species : *V. myrtillus*, *V. oxycoccos*, *V. uliginosum* and *V. vitis-idaea*, as well as *Andromeda polifolia*, *Calluna vulgaris* and *Erica tetralix*) were quantified. These traits included the size of the flower, the number of pollen tetrads and ovules, the distance between the stigma and the anthers, and the exertion of the style. Pollen-ovule ratios suggested a more or less mixed mating system in all species. Floral traits (greater pollen-ovule ratio, style highly exerted, higher stigma-anther distance) in *V. vitis-idaea* suggested that it functioned more as an outcrosser than its congeners. On the opposite, floral traits suggested that *E. tetralix* and *V. uliginosum* appeared to be more selfing than the other species.

KEY WORDS. — Breeding systems, Ericaceae, floral morphology, pollen-ovule ratios.

INTRODUCTION

The comparison of self- and cross-pollination in relation to the functional aspects of floral biology and pollination relationships (LLOYD & SCHOEN 1992) and evolutionary changes in levels of genetic load and inbreeding depression (UYENOYAMA 1986) represents a central concern of plant reproductive biology. An evolutionary shift from predominantly outcrossing to predominantly selfing breeding systems is often correlated with alterations in flower morphology. Among the traits measurable in living specimens, the pollen-ovule ratio (P/O) provides a useful insight into the breeding system of a species (CRUDEN 1977, BOSCH 1992, PRESTON 1986, JÜRGENS *et al.* 2002). Pollen-ovule ratios reflect the pollination efficien-

cy, i.e., the likelihood of a pollen grain reaching the stigma (CRUDEN 1977, 2000). There is a substantial decrease in pollen grain numbers and in P/Os from xenogamy to autogamy. There is evidence that the sexual systems, pollen vectors, pollination mechanisms and ecological factors also affect the P/Os (CRUDEN 2000). Where genetic analyses require extensive time, effort and facilities to provide excellent information, the use of P/Os for the determination of plant breeding systems employs morphological characters that can be assessed quantitatively and is thus intermediate to the modern genetical and the traditional purely morphological approaches (PRESTON 1986). P/Os are employed in comparative studies of related species (FELINER 1991, MIONE & ANDERSON 1992, MOLAU & PRENTICE 1992, RAMSEY 1993, MAZER &

HULTGARD 1993, NG & CORLETT 2000, BOSCH *et al.* 2001, JÜRGENS *et al.* 2002).

Outcrossing plants are often self-incompatible, protandrous, with marked stigma-anther distance, large showy flowers that have relatively long blooming times, and high P/Os (ORNDUFF 1969, CRUDEN 1977, BOSCH 1992). In contrast, self-fertilising taxa often show reduced temporal and spatial separation of male and female functions, flower size and attractiveness, flowering duration and P/Os (CRUDEN 1977, CRUDEN & LYON 1989). In obligately autogamous species, the P/Os should be under much stronger stabilizing selection than in outcrossing taxa (BRUNET & CHARLESWORTH 1995, MAZER & DELESALLE 1998). Consequently, MAZER & DELESALLE (1998) have also predicted that obligately autogamous taxa should exhibit lower genetic coefficients of variation in the P/Os.

There has been little attention given to the floral biology of the group of related species inhabiting peat bogs and wet heath communities in north-west Europe. In such habitats, the Ericaceae family is a significant element: four genera (*Andromeda*, *Calluna*, *Erica* and *Vaccinium*) comprise major components of the perennial shrubs in Belgium. In Europe, the Ericaceae family comprises 18 genera and 50 species (TUTIN *et al.* 1968). Reproduction is both vegetative and by seeds. Species of the Ericaceae display some variation in floral form and traits related to their pollination ecology (WARMING 1908, RITCHIE 1955, 1956, GIMINGHAM 1960, BANNISTER 1966, READER 1977, VANDER KLOET & LYRENE 1987, KREBS & HANCOCK 1990, RATHCKE & REAL 1993, HOKANSON & HANCOCK 2000, KRON *et al.* 2002). The inflorescence varies among genera and species in its structure and number of flowers (see Table 1 and Figs. 1-2). The flowers vary interspecifically with respect to their size, form, anther dehiscence and stigma-anther distance (JACQUEMART 1996, 1997, 1998, FRÖBORG 1996, MAHY *et al.* 1998). Nevertheless, some traits are constant in the studied species: the inflorescence type is in all cases a raceme, the flowers are tetra- or penta-meric, with poricidal anthers (except *C. vulgaris*) and present rewards to the visitors as pollen tetrads and nectar. In all

our studied species, flowers are nectariferous and attracting an array of pollinators and nectar thieves, mainly belonging to the Apidae (bumblebees and honeybees) and the Syrphidae (JACQUEMART 1993, MAHY *et al.* 1998). The corollas are predominantly pink (white to purple). The species are slightly protandrous (or homogamous for *A. polifolia* and *V. vitis-idaea*).

The aim of this paper was to describe the floral morphology of seven Belgian sympatric Ericaceae and to discuss their reproductive systems in relation to these morphological traits.

MATERIALS AND METHODS

SPECIES

Seven taxa were studied: *Andromeda polifolia* L., *Calluna vulgaris* (L.) Hull., *Erica tetralix* L. and the four *Vaccinium* species, which are *V. myrtillus* L., *V. oxycoccus* L., *V. uliginosum* L. and *V. vitis-idaea* L. (see Figs. 1 and 2, Table 1).

Andromeda polifolia L. is an evergreen, tetraploid ($2n = 48$) dwarf shrub (15-35 cm). Its flowers are inclined or pendent, in racemes of 2-7 (12) and present bright pink corollas (TUTIN *et al.* 1968, JACQUEMART 1998).

Calluna vulgaris (L.) Hull. is an evergreen, diploid ($2n = 24$) dwarf shrub (15-80 cm). The flowers are grouped in narrow racemes that are sometimes grouped into panicles. The sepals are oblong, pinkish and longer than the corolla, which is lobed nearly to the base (GIMINGHAM 1960, MAHY & JACQUEMART 1998).

Erica tetralix L. is an evergreen, diploid ($2n = 24$) dwarf shrub (20-70 cm). Its 9-12 flowers are grouped in terminal racemes and present pale pink urceolate corollas (BANNISTER 1966).

Andromeda, *Calluna* and *Erica* possess a capsular fruit with numerous seeds.

The genus *Vaccinium* L. (Vaccinoideae) comprises trees and shrubs with fruits in the form of an edible berry (TUTIN *et al.* 1968, CANE *et al.* 1985).

V. myrtillus L. is a deciduous, diploid ($2n = 24$), clonal (ALBERT *et al.* 2003) dwarf shrub (10-70 cm). The flowers are single or rarely in pairs and present green to purple pendulous urceolate corollas (RITCHIE 1956).

V. oxycoccus L. presents terminal racemes of 1-5 pink open flowers. This species is evergreen, prostrate and tetraploid ($2n = 48$; VANDER KLOET 1983, JACQUEMART 1997).



FIG. 1. — Inflorescence (1) and cross-sections of flowers (2) of the 'capsule' Ericaceae species. (A) : *Andromeda polifolia*, (B) : *Calluna vulgaris* and (C) : *Erica tetralix*.

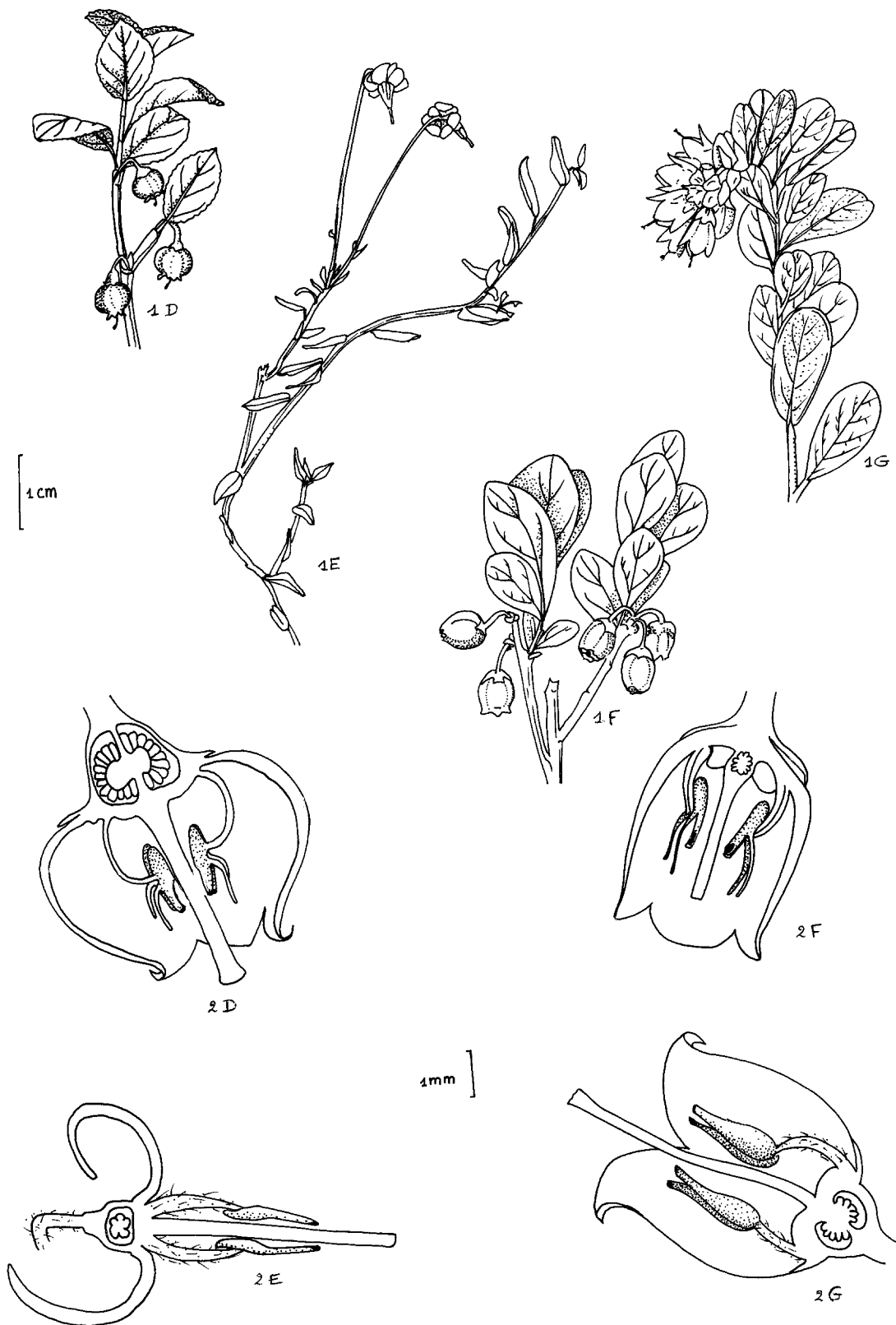


FIG. 2. — Inflorescence (1) and cross-sections of flowers (2) of the four *Vaccinium* species. (D) : *V. myrtillus*, (E) : *V. oxycoccos*, (F) : *V. uliginosum* and (G) : *V. vitis-idaea*.

TABLE 1
Some life form and floral traits of the seven Belgian *Ericaceae*

Character	<i>A. polifolia</i>	<i>C. vulgaris</i>	<i>E. tetralix</i>	<i>V. myrtillos</i>	<i>V. oxycoccus</i>	<i>V. uliginosum</i>	<i>V. vitis-idaea</i>
Sempervirence	Yes	Yes	Yes	No	Yes	No	Yes
Ploidy	2n = 48	2n = 24	2n = 24	2n = 24	2n = 48	2n = 48	2n = 24
Height of the plant (cm)	10-25	15-80	20-70	10-70	<10	40-80	10-30
4- or 5- merous	5	4	4	5	4	4 or 5	4
Number of flowers per inflorescence	2-7	>20	9-12	1-2	1-5	1-4	2-12
Flower type	Urceolate-globose	Campanulate	Urceolate-globose	Urceolate-globose	Reflexed lobes	Urceolate-globose	Campanulate
Flower colour	Pink	Pinkish	Pale pink	Green to purple	Pinkish-red	Greenish-white	White
Fruit type	Capsule	Capsule	Capsule	Berry	Berry	Berry	Berry

TABLE 2

Mean values of floral characters on the seven Belgian *Ericaceae*. Groups that showed significant differences ($P < 0.05$) are indicated with different letters. Unless stated, values are means of 12 measurements (\pm STD) and are in millimetres. P/O (= pollen to ovule ratio) data were arcsine transformed

Character	<i>A. polifolia</i>	<i>C. vulgaris</i>	<i>E. tetralix</i>	<i>V. myrtillos</i>	<i>V. oxycoccus</i>	<i>V. uliginosum</i>	<i>V. vitis-idaea</i>	F
Flower diameter	4.1 \pm 0.4 c	3.8 \pm 0.5 c	3.5 \pm 0.4 c	5.6 \pm 0.2 a	5.1 \pm 0.7 b	3.8 \pm 0.2 c	4.9 \pm 0.3 b	48.286
Flower length	4.2 \pm 0.4 c	3.3 \pm 0.2 d	6.3 \pm 0.3 a	4.7 \pm 0.2 b	4.8 \pm 0.6 b	4.8 \pm 0.2 b	5.0 \pm 0.2 b	73.801
Style exertion	-1.1 \pm 0.2 d	0.4 \pm 0.04 c	0.4 \pm 0.06 c	0.9 \pm 0.2 b	0.7 \pm 0.2 b	-1.0 \pm 0.2 d	1.9 \pm 0.4 a	182.038
Stigma-anther distance	1.9 \pm 0.1 b	1.5 \pm 0.3 c	1.2 \pm 0.1 d	1.9 \pm 0.4 b	0.7 \pm 0.4 e	1.3 \pm 0.2 d	3.1 \pm 0.2 a	157.032
Ovules per flower	46.4 \pm 3.3 d	31.1 \pm 3.3 e	97.1 \pm 10.6 a	90.8 \pm 13.5 b	21.6 \pm 6.2 f	84.9 \pm 6.5 b	64.1 \pm 5.0 c	194.193
Tetrads per anther	402.7 \pm 38.7 e	472.7 \pm 227.8 de	385.4 \pm 83.7 e	1291.7 \pm 290.2 b	781.7 \pm 397.0 c	555.0 \pm 86.6 d	1462.3 \pm 227.3 a	75.905
P/O ratio	347.7 \pm 25.0 de	493.1 \pm 195.8 d	136.7 \pm 40.0 f	582.5 \pm 48.4 c	1666.7 \pm 589.2 a	286.6 \pm 31.1 e	783.8 \pm 80.5 b	56.419
Tetrad diameter (μ m) (50)	46.8 \pm 2.9 b	41.5 \pm 4.8 d	37.9 \pm 3.2 e	46.6 \pm 2.9 b	31.9 \pm 3.3 f	48.3 \pm 2.3 a	45.1 \pm 3.7 c	149.258

V. uliginosum L. is deciduous and tetraploid ($2n = 48$). It has greenish-white pendulous, urceolate, globose corollas. The inflorescences bear 1 to 4 flowers. This species is larger (40-80 cm) than the other *Vaccinium* species studied (WARMING 1908, JACQUEMART 1996).

V. vitis-idaea L. is an evergreen diploid ($2n = 24$) heath shrub. It has a slightly pendulous raceme of 2-12 campanulate white flowers (WARMING 1908, RITCHIE 1955).

STUDY SITE

The study was carried out in 1989 for *V. myrtillus*, *V. uliginosum* and *V. vitis-idaea*, in 1995 for *V. oxycoccus* and *E. tetralix*, in 1996 for *C. vulgaris* and in 2002 for *A. polifolia*. The study site consisted of a heathland and a peat bog dominated by all species investigated. This site is located in the Upper Ardenne, Belgium (N 50°15'00", E 5°44'22", alt. 652m). Investigations extended from mid April until mid September.

FLORAL MORPHOLOGY

Just before flower opening, twelve mature flower buds (each bud sampled from a different individual) were randomly collected from each of the species and preserved in 70% alcohol. These buds were dissected under a stereo-microscope to count the number of ovules and pollen tetrads were counted under a light microscope. From the pollen and ovule production, pollen-ovule ratio (P/O) per flower was calculated. Pollen size measurements (tetrad diameter) were performed by light microscopy. Other floral traits were measured on 12 mature fresh flowers (one day old) of each species. Each mature flower was sampled from the same individuals as the corresponding buds. Flower diameter and length, style and stamen length, and style exertion were measured using digital callipers (to 0.01 mm). Flower length corresponds to the longest dimension from the base of the flower to the tip of the petals. Style exertion corresponds to the dimension from the stigma to the end of the corolla (or the end of the anthers for *V. oxycoccus*). Stigma-anther distance was calculated by subtracting style length from stamen length.

DATA ANALYSIS

Floral traits were compared statistically using one-way analysis of variance (ANOVA) with Fisher contrasts using SAS (SAS INSTITUTE 1993). Pollen-ovule ratios (P/Os) were arcsine transformed prior to being analysed.

RESULTS

There was considerable variation among species in the number of ovules and pollen tetrads (Table 2). *Erica tetralix* presented the highest number of ovules (97 ± 11) and the smallest number of tetrads (385 ± 84), resulting in the lowest P/O observed (137). On the other hand, the smallest number of ovules was found in *V. oxycoccus* (22 ± 6) with an intermediate and variable number of tetrads (782 ± 397), resulting in the highest P/O (1667) of all the species tested. The highest number of tetrads was found in *V. vitis-idaea* (1462 ± 227) with an intermediate number of ovules (64 ± 5) and a quite high P/O (784).

The sizes of pollen grains were also different: *V. uliginosum* presented the biggest tetrads ($48 \mu\text{m}$) and the smallest were those of *V. oxycoccus* ($32 \mu\text{m}$).

Several other floral traits were also significantly different among species. *Vaccinium myrtillus* presented the widest flowers (5-6 mm), the smallest ones were those of the other genera: *Erica*, *Andromeda* and *Calluna* (3-4 mm). Within these species, *E. tetralix* presented the longest flowers (6 mm), the shortest ones were those of *C. vulgaris* (3-4 mm). The length of the flower was similar among all the *Vaccinium* species (4.7-5.0 mm).

The style of *V. vitis-idaea* was the most exerted (1.9 mm), followed by the style of *V. myrtillus* and *V. oxycoccus*, the stigma of the other species was more or less near the throat of the corolla or the bottom of the anthers (*V. oxycoccus*). Only in two species, *V. uliginosum* and *A. polifolia*, the style was included in the corolla. The largest distance between stigma and anthers was found with the exerted style of *V. vitis-idaea* and the smallest (but variable) value was that of *V. oxycoccus*. The distances were highly variable among the *Vaccinium* species.

DISCUSSION

All our morphological data were similar to those of other studies (WARMING 1908, RITCHIE 1955, 1956, PAQUEREAU 1959, TUTIN *et al.* 1968, BARANEC *et al.* 1996). Except *V. oxycoccus*, P/Os

of the studied Ericaceae fall within the range that, according to CRUDEN (1977), characterises mixed mating species (about 797 for facultative xenogamy and 168 for facultative autogamy). Other European Ericaceae exhibited more or less similar mixed trends, e.g. *Erica cinerea* (BANNISTER 1966) or *Rhododendron ferrugineum* L. (ESCARAVAGE *et al.* 1997). American Ericaceae species are also considered as facultatively xenogamous, sometimes more or less self-incompatible or suffering of early-acting inbreeding depression (READER 1977, VANDER KLOET & LYRENE 1987, KREBS & HANCOCK 1990, RATHCKE & REAL 1993, HOKANSON & HANCOCK 2000). Moreover, the P/Os were studied in some other Ericaceae species, e.g. six species of *Rhododendron* from Hong Kong China that exhibited a similar range of P/Os, from 176 in *R. simsii* to 1343 in *R. simiarum* (NG & CORLETT 2000).

FLORAL TRAITS AS INDICATORS OF BREEDING SYSTEMS

Clear-cut differences between the studied species occurred in some floral traits, namely stigma-anther distance, style exertion and P/O ratio. Two major groups of species could be distinguished based on these morphological traits and on the results from pollination experiments.

1. Outcrossing to mixed mating species

For the floral traits, *V. vitis-idaea* had values that suggest a floral phenotype more adapted to cross-pollination than the other species. Of particular importance here is the increased stigma-anther distance, which has often been found to markedly decrease autonomous selfing (BARRETT 1989). The flowers of *V. vitis-idaea* are diversely oriented, the styles are exerted and the anthers are awnless. This suggests that 1) the pollen release strategy is different than in *V. myrtillus* and *V. uliginosum*, i.e. buzz pollination (BUCHMANN 1983) does not occur, and 2) there is less spontaneous selfing due to the pendulous nature of the flowers. Stigma-anther distance and style exertion in *V. vitis-idaea* fit with a generally accepted cross-pollination syndrome (ORNDUFF 1969, CRUDEN & LYON 1989). The P/O was in the range of facultative xenogamous species (CRUDEN

1977). Following hand pollination experiments, cross-pollination yielded more fruits and more seeds than self-pollination (FRÖBORG 1996, GUILLAUME & JACQUEMART 1999, see Table 3). *Vaccinium vitis-idaea* could thus be considered as facultative xenogamous species and its floral traits were in concordance with the results from pollination experiments. In this case, the floral traits could be considered as good indicators for the breeding system.

On the other hand, even with the highest P/O ratio of all the species studied, cross- and self-pollinations yielded the same proportion of fruits in *V. oxycoccos* (Table 3, and see FRÖBORG 1996, DELBUSCHÈCHE, 1996). However, crossed fruits contained more seeds than selfed fruits (DELBUSCHÈCHE, 1996, JACQUEMART, unpublished data). In general, effects of self- versus cross-pollination were less detrimental to fruit set than to seed set since fruits were able to develop despite a very low number of seeds per fruit (FRÖBORG 1996, GUILLAUME & JACQUEMART 1999). According to the floral traits and the seed set results, the species can be assessed to have a mixed mating system or to be a facultative xenogamous species, like *V. vitis-idaea*, even if more data are needed.

The P/Os for *C. vulgaris*, *V. myrtillus*, and *A. polifolia* were lower (348 to 583), as were their stigma-anther distances. However, for *C. vulgaris* and *V. myrtillus*, cross-pollinations yielded more fruits and more seeds than self-pollinations (Table 3, MAHY & JACQUEMART 1999, GUILLAUME & JACQUEMART 1999). Estimations of the outcrossing rates by means of allozymes showed a mean of 66-75% of xenogamy for *V. myrtillus* (JACQUEMART *et al.* 1994) and as high as 70-90% for *C. vulgaris* (MAHY & JACQUEMART 1998). Contrary to *C. vulgaris* and *V. myrtillus*, cross- and self-pollinations yielded the same proportion of fruits in our experiments with *A. polifolia* (Table 3). However, FRÖBORG (1996) observed higher fruit set following cross- versus self-pollinations. Moreover, as well in our experiments (SCHMITZ 2001, JACQUEMART unpublished data) as in FRÖBORG (1996), crossed fruits presented more seeds than selfed fruits. These experiments and estimations were in agreement with the

TABLE 3

Fruit and seed sets following hand cross- and self- pollinations for the seven *Ericaceae* species. The significant levels after analysis of variance (data arcsine transformed) are as follows: NS : non significant; * < 0.05; ** < 0.001; *** < 0.0001. Number of flowers (for fruit set) and of fruits (for seed set) are given in brackets

Treatments	<i>A. polifolia</i>	<i>C. vulgaris</i>	<i>E. tetralix</i>	<i>V. myrtilus</i>	<i>V. oxycoccus</i>	<i>V. uliginosum</i>	<i>V. vitis-idaea</i>
Fruit set							
Crossing	85.6 ± 30.7 (36)	97.8 ± 4.4 (55)	83.4 ± 22.9 (20)	78.7 ± 14.0 (150)	40.0 ± 28.5 (55)	27.7 ± 9.3 (150)	66.0 ± 2.8 (100)
Selfing	87.9 ± 30.8 (36)	46.8 ± 24.1 (55)	87.2 ± 25.0 (20)	64.0 ± 9.2 (150)	36.3 ± 28.7 (51)	27.3 ± 11.2 (150)	44.5 ± 13.3 (80)
Significance level	NS	**	NS	*	NS	NS	**
Seed set							
Crossing	69.6 ± 12.9 (12)	14.9 ± 6.5 (54)	69.7 ± 5.0 (2)	40.3 ± 19.6 (100)	71.5 ± 17.4 (12)	5.7 ± 5.0 (35)	51.5 ± 15.9 (86)
Selfing	26.2 ± 13.3 (12)	2.0 ± 1.5 (23)	56.4 ± 20.6 (11)	10.2 ± 3.1 (100)	37.2 ± 14.5 (12)	8.0 ± 6.0 (50)	14.3 ± 2.1 (42)
Significance level	***	***	NS	***	***	NS	***
Reference number	6, 7	2, 4	3	1, 5	3, 7	1	1, 5

Data from (1) JACQUEMART & THOMPSON 1996, (2) BRUNEL 1996, (3) DELBUSCHÈCHE 1996, (4) MAHY & JACQUEMART 1998, (5) GUILLAUME & JACQUEMART 1999, (6) SCHMITZ 2001, and (7) unpublished data.

facultatively xenogamous classification of these three species.

2. Mixed mating to selfing species

On the opposite, two species seemed to be more selfing than all those species. *Erica tetralix* and *V. uliginosum* presented floral traits (P/Os, stigma-anther distances, exertion of styles) suggesting that they are likely to self to a greater extent and could be classified as facultatively autogamous according to CRUDEN's (1977) classification. The P/O of *E. tetralix* was the lowest one of all species. Accordingly, cross- and self-pollinations yielded the same proportions of fruits and, in these two species, the same proportions of seeds (Table 3, and see JACQUEMART & THOMPSON 1996, BRUNEEL 1996, DELBUSCHÈCHE 1996).

In conclusion, P/Os, stigma-anther distance and exertion of the style give quite good estimates of the breeding system for several species in the Ericaceae family (except perhaps for *V. oxycoccus*). P/Os correspond quite well to CRUDEN's categories because no important deviations may influence their values in this case (PRESTON 1986) : species are insect-pollinated, offer both nectar and pollen and are hermaphroditic. Except *E. tetralix* and *V. uliginosum* that were mainly selfing, all the other species exhibited a mixed mating system. Further data are needed on more species for a better understanding of the main factors affecting the breeding system in the Ericaceae family.

A similar congruence between P/Os and pollination experiments was observed in comparing five mixed mating *Ranunculus* species (PICKERING 1997), for the Cruciferae (PRESTON 1986, MIONE & ANDERSON, 1992) or for the Caryophylloideae (JÜRGENS *et al.* 2002). On the other hand, wind-pollination, pollen reward alone and other breeding systems could result in a much higher P/Os than those indicated by CRUDEN (see PRESTON 1986). On the opposite, too low P/Os were observed in *Rhododendron* (NG & CORLETT 2000) mainly due to the unusual packaging of pollen that may allow efficient transfer of substantial loads to pollinators and reduce wastage, thus reducing the need for a large excess of pollen. P/Os were estimated as not appropriate, for exam-

ple, in the case of the genus *Pandorea*, Bignoniaceae (JAMES & KNOX 1993), in the tribe Delphinieae, Ranunculaceae (BOSCH *et al.* 2001) or in Asclepiadaceae (WYATT *et al.* 2000).

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