

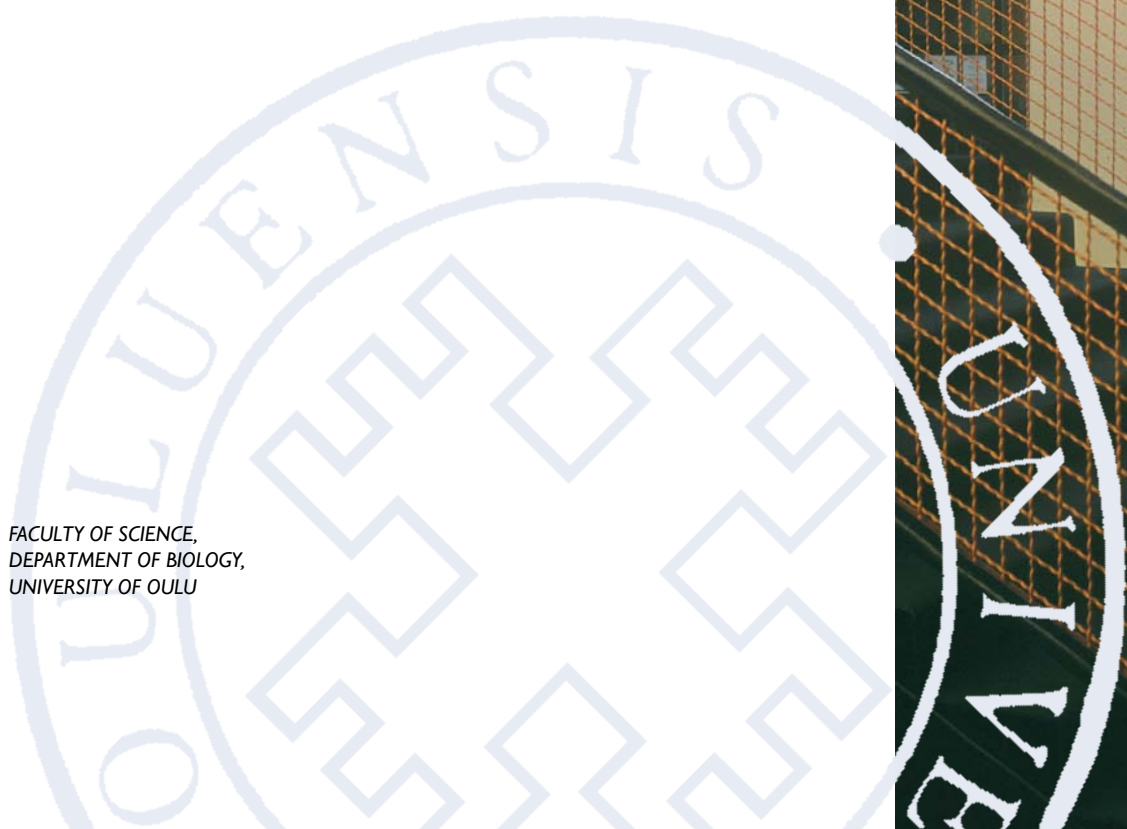
Carolyn Nuortila

CONSTRAINTS ON SEXUAL
REPRODUCTION AND SEED
SET IN *VACCINIUM* AND
CAMPANULA

FACULTY OF SCIENCE,
DEPARTMENT OF BIOLOGY,
UNIVERSITY OF OULU

A

SCIENTIAE RERUM
NATURALIUM



ACTA UNIVERSITATIS OULUENSIS
A Scientiae Rerum Naturalium 489

CAROLIN NUORTILA

**CONSTRAINTS ON SEXUAL
REPRODUCTION AND SEED SET
IN *VACCINIUM* AND *CAMPANULA***

Academic dissertation to be presented, with the assent of
the Faculty of Science of the University of Oulu, for public
defence in Kuusamonsali (Auditorium YB210), Linnanmaa,
on June 15th, 2007, at 12 noon

OULUN YLIOPISTO, OULU 2007

Copyright © 2007
Acta Univ. Oul. A 489, 2007

Supervised by
Docent Kari Laine
Professor Juha Tuomi

Reviewed by
Professor José Ramón Obeso
Docent Veikko Salonen

ISBN 978-951-42-8499-1 (Paperback)
ISBN 978-951-42-8500-4 (PDF)
<http://herkules.oulu.fi/isbn9789514285004/>
ISSN 0355-3191 (Printed)
ISSN 1796-220X (Online)
<http://herkules.oulu.fi/issn03553191/>

Cover design
Raimo Ahonen

OULU UNIVERSITY PRESS
OULU 2007

Nuortila, Carolin, Constraints on sexual reproduction and seed set in *Vaccinium* and *Campanula*

Faculty of Science, Department of Biology, University of Oulu, P.O.Box 3000, FI-90014 University of Oulu, Finland

Acta Univ. Oul. A 489, 2007

Oulu, Finland

Abstract

Plant reproductive success is affected by a number of factors, such as climatic conditions and plant resource status during flowering and fruiting, and pollen origin in fertilization. In the present thesis project, different aspects of plant reproductive ecology were investigated in order to identify constraints on sexual reproduction and seed set in two clonal dwarf shrubs (*Vaccinium myrtillus* and *V. vitis-idaea*) and one long-lived perennial herb (*Campanula rotundifolia*). The work comprised phenological observations and experiments with the clonal shrubs at natural boreal forest sites in the Oulanka National Park in northern Finland. The impact of mycorrhiza on *C. rotundifolia* fitness traits was tested in hand pollinations in a greenhouse experiment.

Pollen origin had some effect on fruit set, and had strong effects on the number of matured seeds in all three species. Seed yield reductions upon hand self-pollination as compared with hand cross-pollination were attributed to inbreeding depression in *V. myrtillus*, and presumably to partial self-incompatibility in *C. rotundifolia*. *V. myrtillus* and *V. vitis-idaea* showed a population structure where the number of matured seeds per fruit increased with increasing distance between pollen donor and pollen recipient. Clonal growth in concert with the foraging behaviour of bumblebee pollinators is thought to cause the possibility of either uniparental or biparental inbreeding, with a strong effect on the number of matured seeds per berry.

In a flower-removal experiment lasting three years, costs of fruiting to future fecundity and vegetative traits were observed, but not to future survival in *V. vitis-idaea* and *V. myrtillus*. The response was more pronounced in the evergreen than in the deciduous species. In *Campanula rotundifolia*, mycorrhiza was associated with a cost to the plants' reproductive effort, as plant biomass and the number of flowers produced per plant were decreased in mycorrhizal plants in comparison with non-mycorrhizal plants. There was no difference in seed number, seed weight or germination between the seeds of mycorrhizal and non-mycorrhizal plants. However, the offspring of mycorrhizal plants had a higher relative growth rate, while also having a higher seed phosphorus concentration.

In summary, sexual reproduction was variably constrained by previous reproduction in *Vaccinium*, and contrary to expectations, by mycorrhiza in *Campanula*. However, mycorrhiza had positive effects on some measures of offspring fitness. In all three species, self-pollination limited seed production.

Keywords: autofertility, *Campanula*, Ericaceae, flowering phenology, inbreeding depression, reproductive success, self-fertility, *Vaccinium*

Acknowledgements

This study has been conducted in the Department of Biology at the University of Oulu. The field work has been carried out at the Oulanka Research Station (former Oulanka Biological Station). I am most grateful to my supervisors Prof. Juha Tuomi and Doc. Kari Laine for stimulating discussions and encouragement, and for shaping this thesis. Directors Juha Viramo (retired) and Pirkko Siikamäki and the staff at the Oulanka Research Station are warmly thanked for all practical help with the field work. I am very grateful to my co-authors Anne Tolvanen, Minna-Maarit Kytöviita and Jouni Aspi for their cooperation, for sharing their expertise and their opinions. I appreciate highly the help of Pasi Rautio and Pia Mutikainen who instructed me on statistics and who discussed several manuscripts with me, as did also Prof. Jari Oksanen, Kalle Hellström and Outi Manninen. I would like to express my sincerest thanks to Doc. Veikko Salonen and Prof. José Ramón Obeso for reviewing this work, and to Prof. Satu Huttunen for kindly taking care of the official arrangements during the end of this project.

My cordial thanks go to the people who helped during the field and laboratory work and made the practical work so very enjoyable: Esa Härmä, Harri Hautala, Riitta Ryömä, Taina Hanhimäki, Melissa Mayhew, Peter Dunlop, Tellervo Oikarinen and several more people at Oulanka Research Station, and Sanna Toivanen, Tarja Törmänen, Anna-Liisa Ruotsalainen, Jukka Kukkonen and Tuulikki Pakonen in the Department of Biology in Oulu. Additional thanks are due to Riitta Blomqvist, Toni Kytöviita, Anna-Liisa Näsilä and Hannelore Nuortila for helping us with the *Campanula* experiment.

Keith Kosola revised the English of the summary; Sirkka-Liisa Leinonen and Helena Power revised the publications and manuscripts, respectively. Their work is greatly acknowledged.

My warmest thanks are due to my mother Hannelore, to my brother Cristian and to my late father Yrjö Nuortila for their continuous support and care.

This project was financially supported by the University of Oulu, The Finnish Cultural Foundation, Suomalainen Konkordia-liitto, Oskar Öflunds Stiftelse, the Academy of Finland and the Graduate School in Evolutionary Ecology, all of which are greatly acknowledged. The Finnish Zoological and Botanical Publishing Board, the NRC Research Press, and the New Phytologist kindly granted permission to reproduce the published articles.

Oulu, May 2007

Carolin Nuortila

List of original papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

- I Nuortila C. Little variation in fruit/flower ratio between years in two ericaceous dwarf shrubs, *Vaccinium myrtillus* and *Vaccinium vitis-idaea*. Manuscript.
- II Nuortila C, Tuomi J & Tolvanen A. Cost of sexual reproduction on future reproduction and vegetative growth in evergreen *Vaccinium vitis-idaea* and deciduous *Vaccinium myrtillus*. Manuscript.
- III Nuortila C, Tuomi J, Aspi J & Laine K (2006) Early-acting inbreeding depression in a clonal dwarf shrub, *Vaccinium myrtillus*, in a northern boreal forest. *Annales Botanici Fennici* 43: 36-48.
- IV Nuortila C, Tuomi J & Laine K (2002) Inter-parent distance affects reproductive success in two clonal dwarf shrubs, *Vaccinium myrtillus* and *Vaccinium vitis-idaea* (Ericaceae). *Canadian Journal of Botany* 80: 875-884.
- V Nuortila C, Kytöviita M-M & Tuomi J (2004) Mycorrhizal symbiosis has contrasting effects on fitness components in *Campanula rotundifolia*. *New Phytologist* 164: 543-553.

Contents

Abstract	
Acknowledgements	5
List of original papers	7
Contents	9
1 Introduction	11
1.1 Plant mating system traits	11
1.2 Cost of reproduction.....	12
1.3 Plant reproduction in response to interaction with environment.....	13
1.4 Aims of the study	15
2 Material and methods	19
2.1 Study area.....	19
2.2 Experimental design.....	19
2.2.1 Phenological observations and natural reproductive success in <i>Vaccinium</i>	19
2.2.2 Manipulating fruiting effort in <i>Vaccinium</i>	20
2.2.3 Conducting hand pollination experiments with <i>Vaccinium</i> and <i>Campanula</i>	21
2.2.4 Greenhouse experiment employing mycorrhizal inoculation in <i>Campanula</i>	22
2.2.5 Statistical analyses.....	23
2.3 Autofertility and self-fertility in Ericaceae	23
3 Results and Discussion	25
3.1 Natural reproductive success in <i>Vaccinium</i>	25
3.2 Cost of reproduction in <i>Vaccinium</i>	27
3.3 Reproductive success in hand pollinated <i>Vaccinium</i> and <i>Campanula</i>	30
3.4 Plant reproduction in response to plant-pollinator interaction	33
3.5 Plant reproduction in response to plant-fungus interaction.....	36
3.6 Autofertility and self-fertility in Ericaceae	38
4 Conclusions	41
References	43
Appendix 1	53
Original papers	55

1 Introduction

Plant reproductive ecology studies the interactions of a sexually reproducing plant with its abiotic and biotic environments. Since plants are sessile, to accomplish mating they usually depend on vectors for pollen transport. Although the majority of flowering plants are hermaphrodites, having both male and female sexual organs in the same flower, not all species are able to pollinate themselves autonomously. Plants have developed a wide array of variable flower morphologies and different reproductive strategies (Richards 1997, Geber *et al.* 1999), and in many cases specialized relationships with their pollinators.

In sexual reproduction, plant genetic material is recombined and genetically variable offspring are created (Stearns & Hoekstra 2000). Moreover, propagules are formed for dispersal that enables plants to colonize new habitats. The number of seeds produced by plants is determined in the first place by the number of ovules per ovary and the number of flowers per plant. Seed production can be constrained by insufficient or inadequate pollination, resource restrictions of the maternal plant, and relatedness of mating partners.

1.1 Plant mating system traits

Plant mating systems are determined by those features that affect gene inheritance from one generation to another (Wyatt 1983). Different plant species exhibit a wide variety of floral traits that influence the pattern of mating between plants. Mating systems are basically distinguished by the occurrence of female and male reproductive organs in the same or in different plant individuals (dioecy or cosexuality), or in the same or different flowers (hermaphroditism or monoecy) (Richards 1997). In plants with hermaphrodite flowers, the reproductive organs can furthermore be separated in space (herkogamy) or they can function at different times (dichogamy). All of these traits have the capacity to influence the occurrence of self-pollination or cross-pollination, as has self-incompatibility, the genetically determined rejection of self-pollen (Barrett 1988, Richards 1997). Furthermore, mating systems can be characterized by a plant's ability to autonomously self-pollinate and to fertilize upon experimental self-pollination as compared with cross-pollination (cf. Lloyd & Schoen 1992). The outcome of self-pollination may vary between populations and individual plants in reflection of differences in levels of genetic load, and thus inbreeding depression, or differences in the strength of self-incompatibility systems. In some plant species,

the expression of self-incompatibility has been reported to vary with respect to flower development (Stephenson *et al.* 1992, Vogler *et al.* 1998, Goodwillie *et al.* 2004, Travers *et al.* 2004) and possibly resource-associated conditions, too (Becerra & Lloyd 1992, Reinartz & Les 1994).

In plant reproductive biology, special emphasis is placed on evaluating the fitness consequences of self-fertilization and cross-fertilization. Since self-fertilization is often associated with detrimental effects on progeny fitness due to genetic load, inbreeding depression has been regarded as the basic driving force in the evolution of plant mating systems (Lande & Schemske 1985, Charlesworth & Charlesworth 1987, Holsinger 1991). Theoretical considerations have predicted a bimodal distribution of predominantly selfing and predominantly outcrossing mating systems in plants (Lande & Schemske 1985), and this prediction has been confirmed in a review on natural species (Schemske & Lande 1985). According to this model, intermediate selfing rates should not be evolutionarily stable. However, this notion has been questioned by an increasing number of reports on species with mixed mating systems (Goodwillie *et al.* 2005).

Barrett *et al.* (1996) compared the frequency of self-fertilizing plant species with respect to their growth form and concluded that woody perennial species are less frequently self-fertilizing than are herbaceous perennials or annuals. Moreover, inbreeding depression appears to be stronger in woody perennial species than in annuals, due to higher mutation rates per generation in the longer-lived species (Klekowski 1988, Klekowski & Godfrey 1989). Nevertheless, in many plant species, the outcrossing or selfing rate is subject to variation due to genetic, demographic or environmental factors (*e.g.* Barrett & Eckert 1990). Moreover, environmental conditions also seem to affect the expression of inbreeding depression in a number of organisms (Dudash 1990, Armbruster & Reed 2005).

1.2 Cost of reproduction

During sexual reproduction plants allocate resources to the production of flowers, fruits and seeds, and their supportive structures. A central hypothesis in resource allocation theory is the one of cost of reproduction (Stearns 1989, Roff 1992). This hypothesis is based on the assumption that there is a restricted pool of resources that a plant can utilize for growth or reproduction. Consequently, if reproduction competes more or less directly for the same limited resources, investment in reproduction will diminish the resources available for future

growth, reproduction and survival (Stearns 1989). But, even though there may be potential costs, they might not be detected in situations when *e.g.* plants are able to assimilate more in order to cover higher demands during flowering and fruiting (Reekie & Bazzaz 1987). Thus, it has been assumed that there might be a threshold beyond which plant investments in reproduction have to grow before costs become visible (Tuomi *et al.* 1983). On the other hand, costs may be conditional and therefore expressed more strongly or exclusively when plants are subjected to stress, like when growing in low soil resource conditions (Biere 1995), or when being affected by herbivory (Primack *et al.* 1994). Also, responses of the same species investigated in different habitats or study sites have been reported to be variable (*e.g.* Obeso 1993a, Syrjänen & Lehtilä 1993, Hemborg 1998).

Literature on the cost of reproduction mostly reports individual species' responses. So far, it seems that no general trends have been identified as to whether costs of reproduction are expressed more often in some life history traits than in others, or whether taxonomically related species or cosexual plants with the same growth form express costs in similar traits (but see Obeso 2002). Assumedly, in long-lived perennial species, fecundity costs are more common than survival costs, whereas survival costs could be more common in short-lived species. Life-history models assume that short-lived species are highly dependent on predictable juvenile survival whereas long-lived species are dependent on predictable adult survival. Therefore, to be able to detect costs of reproduction in long-lived plants, it is important to conduct experiments over more than one season (Primack & Hall 1990, Ehrlén & van Groenendael 2001).

1.3 Plant reproduction in response to interaction with environment

Plant reproductive success can be affected by *e.g.* pollinator services (Bierzychudek 1981, Burd 1994), predation of flowers, fruits and seeds (Herrera 1993, García *et al.* 2000), herbivory (Hendrix 1988), defoliation (Obeso 1993b) and resource levels (Campbell & Halama 1993, Lau & Stephenson 1993, Helenurm & Schaal 1996). Nutrient limitations have been demonstrated to reduce flower, fruit and seed production in many species (*e.g.* Helenurm & Schaal 1996). Moreover, pollen number and quality can be affected by changes in resource levels of the pollen-producing plant, as occurs in herbivory, fertilization or mycorrhizal symbiosis (Lehtilä & Strauss 1999; see also reviews by Mutikainen & Delph 1996, Delph *et al.* 1997).

In general, plant nutrient acquisition is affected in a positive way in mycorrhizas, and a number of laboratory studies have reported increased reproductive capacity (flower number, pollen traits) in mycorrhizal plants (Koide *et al.* 1988, Stanley *et al.* 1993, Koide 2000, Poulton *et al.* 2001). Both nutrient addition (Lau & Stephenson 1993, cf. review by Delph *et al.* 1997) and mycorrhiza (Poulton *et al.* 2001, 2002, Philip *et al.* 2001) have been shown to *e.g.* influence pollen production and pollen tube growth, and therefore, a positive effect of mycorrhiza on plant male function measured as seed siring success seems possible. However, so far no attention has been paid to whether mycorrhiza is capable of affecting reproductive success differentially in controlled hand self-pollinations and cross-pollinations.

Environmental conditions may potentially mediate plant mating systems, too, by differentially affecting the abortion of embryos from self-fertilizations and cross-fertilizations. Nutrient limitations of maternal plants have been shown to reduce the relative success of self-fertilized seeds as compared with cross-fertilized seeds (Hill-Cottingham 1967, Martin & Lee 1993, Helenurm & Schaal 1996, Levri & Real 1998). Levri (1998) gave a number of possible explanations for the higher abortion of selfed seeds late in the season in *Kalmia latifolia*, such as changes in resource availability during the flowering season, with effects on seed maturation, pollen quality or pistil receptivity. In *Lupinus texensis*, the result was discussed in the context of inbreeding depression being more strongly expressed during seed development under stressful conditions (Helenurm & Schaal 1996). Martin & Lee (1993) reasoned that abortion of selfed seeds in *Cassia fasciculata* in nutrient-limited conditions may take place when the genetic load is composed of inferior but nonlethal gene combinations.

Plant populations tend to be genetically structured. Because seed dispersal in space is rather limited, plants are commonly genetically more related to their neighbour plants than to far-distant plants (Handel 1983, Silvertown & Charlesworth 2001). Unless diaspores are specialized, seeds are released into the immediate surroundings of the mother plant, thus creating patches of genetically similar plants. Moreover, mating is more likely to occur between spatially close plants than between very distant plants, especially in insect-pollinated plant species (Schmitt 1980, Waddington 1983). If inter-plant distance is inversely associated with genetic similarity, the distance between pollen donor and pollen recipient assumedly has an effect on reproductive success or offspring fitness in self-incompatible plants or plants suffering from inbreeding depression (Levin 1984, 1989, Dudash 1990, Trame *et al.* 1995). On the other hand, environmental

conditions may give rise to local adaptation, and consequently, mating between plants that are separated by a longer distance can lead to a disruption of those favourable adaptations (Price & Waser 1979, Schemske & Pautler 1984, Waser & Price 1989). In this case, reproductive success or fitness of offspring might be lower than in matings between plants inhabiting the same habitat or site, an outcome that is called outbreeding depression.

Clonality is another factor leading to the genetic structure in plant populations. In clonal plants each new ramet produced is basically a replicate unit of the same genotype. With increasing clonal growth the genotype occupies a larger area with the effect that ramets are probably neighboured by ramets belonging to the same genet. Pollen flow has a strong impact on quality and perhaps also the quantity of reproductive success in plants in general (*e.g.* Levin 1981), and assumedly in clonal plants in particular. Geitonogamy, or self-pollination between flowers within the same plant, is expected to increase with plant size (de Jong *et al.* 1993), and pollinator foraging between different ramets may lead to additional self-pollination in clonal plants (Eckert 2000). Handel (1985) hypothesized that the mating system of individual clonal plants may be affected during growth, because the likelihood of geitonogamy increases as the clone grows.

1.4 Aims of the study

In order to evaluate constraints on sexual reproduction, I investigated aspects of reproductive success, mating system traits and costs of reproduction in two clonal dwarf shrubs, *Vaccinium myrtillus* L. (bilberry) and *V. vitis-idaea* L. (lingonberry), Ericaceae, and one long-lived perennial, *Campanula rotundifolia* L. (harebell), Campanulaceae. In particular, between-year variation in flowering intensity and fruit and seed production, fruit and seed maturation in response to different types of experimental hand pollinations, and the impact of nutritional status of the maternal plant on seed number and offspring quality were investigated (Table 1). Moreover, I prepared a short review to compare the present results with the reproductive features of other ericaceous species.

V. myrtillus and *V. vitis-idaea* are clonal dwarf shrubs that dominate the understorey in boreal forests. They grow extensively with the help of rhizomes. From the belowground stem, deciduous *V. myrtillus* produces aerial shoots (ramets) that are about 30 cm in height, and the evergreen *V. vitis-idaea* produces ramets of about 10 to 30 cm height, depending on the habitat (Popova 1972).

Both species flower and produce berries regularly. In *V. myrtillus*, pentamerous flowers are produced in reduced racemes of 1 to 2 flowers. *V. vitis-idaea* produces inflorescences of 3-15 tetramerous flowers. The flowers of both species have anthers that are elongated into two tubes and that open with a small pore. Pollen is released in tetrads. Both species produce nectar and they are insect-pollinated (Knuth 1899, Ritchie 1955, 1956, Jacquemart 1993). However, previous studies on *Vaccinium* species have pointed out that there is a paradox of abundant berry production when at the same time seeds appear to be rare in seed banks (Vander Kloet & Hill 1994, Welch *et al.* 2000). *Vaccinium* species form ericoid mycorrhiza (Harley & Harley 1987). Furthermore, *V. myrtillus* and *V. vitis-idaea* are among the most important wild berry plants with commercial significance in Finland. From 1977 to 2005, a yearly average of 2.1 million kilograms of bilberries were picked in the whole country and they provided a yearly average picking income of 2.3 million euros (Finnish Forest Research Institute 2006). During the same period, on average 4.7 million kilograms of lingonberries were picked and they contributed an average picking income of 4.9 million euros each year.

In the present study, the practical work comprised phenological observations (I), and manipulative experiments (II-IV) with *V. myrtillus* and *V. vitis-idaea* at natural forest sites and in an experimental field in northern Finland. *Vaccinium* flowering phenology, fruit set and seed data were recorded from the same sites over several years to estimate variation in natural reproductive success (I). The costs of reproduction were investigated by manipulating fruit set (II). The basic features of the mating system were investigated in several hand pollination experiments with *V. myrtillus* (III, IV) and *V. vitis-idaea* (IV). In order to investigate whether insect-pollinated *V. myrtillus* and *V. vitis-idaea* showed a distance-related genetic structure in the populations, hand pollinations were conducted where the distance between pollen-donor and pollen-recipient increased (IV). In this work, pollinator-flight distances during foraging on *V. myrtillus* and *V. vitis-idaea* were also recorded. Approaches I-IV were taken to assess whether *Vaccinium* species have features in their reproductive biology that promote cross-pollination.

In the last work, we investigated whether mycorrhiza had the potential to affect a species' vegetative performance, sexual reproduction, mating system, and offspring quality (V). Since mycorrhizal effects on plant fitness characters can only be investigated under controlled conditions, a herbaceous study system was chosen. A greenhouse experiment was started from seed material of *Campanula*

rotundifolia and employed inoculation with mycorrhizal fungi. *C. rotundifolia* is a common perennial throughout northern Europe. The species has bell-shaped light blue flowers that are hermaphroditic and protandrous, with secondary pollen presentation (Fægri & van der Pijl 1979, Nyman 1992, 1993). The family of Campanulaceae is heavily mycorrhizal (Harley & Harley 1987), and *C. rotundifolia* has been found to form mycorrhiza under experimental conditions (Wijesinghe *et al.* 2001, Kytöviita *et al.* 2003).

Table 1. Constraints on reproductive traits as investigated in the present thesis. The original papers with the investigated traits are referred to by Roman numerals.

Reproductive phase and investigated traits	Constraints
Flowering	
Phenology onset and duration	climatic conditions: between-year variation (I)
Number of flowers	resource availability: cost of reproduction (I, II); nutritional status of maternal plant (V)
Fruit maturation	
Number of matured fruits; fruit set percentage	climatic conditions: between-year variation (I) resource availability: cost of reproduction (I, II); nutritional status of maternal plant (V) pollinator services: visitation frequencies (I) pollen origin: self-incompatibility, inbreeding depression (III, IV, V)
Seed maturation	
Number of mature seeds / fruit	climatic conditions: between-year variation (I) resource availability: nutritional status of maternal plant (V) pollen origin: self-incompatibility, inbreeding depression (III, IV, V)
Seed germination	
Time to germination; percentage of germination	seed provisioning: nutritional status of maternal plant (V) genetic load: inbreeding depression (V)

2 Material and methods

2.1 Study area

V. myrtillus and *V. vitis-idaea* were investigated in field experiments (I-IV) conducted in the Oulanka National Park (66°20'N, 29°20'E) in northern Finland. *V. myrtillus* was investigated in spruce-dominated mesic heath forests and *V. vitis-idaea* in moderately dry pine-dominated forests. The exact size of individual *V. myrtillus* and *V. vitis-idaea* clones in the study area is unknown. Both species grow rather uniformly throughout the field layer without forming distinct patches. Patch forming has been reported for *V. myrtillus* at sites in Western Europe. *V. myrtillus* was also examined in an experimental field that had been constructed in 1993 in the close vicinity of the Oulanka Research Station (III). Pieces of *V. myrtillus* clones were excavated from the forest and transplanted into the experimental field. Each piece of clone consisted of at least one ramet and some tens of centimeters of rhizome. The clones were allowed to grow for three years before pollination experiments were conducted. Experiments with *Campanula rotundifolia* were conducted under greenhouse conditions in the Department of Biology at the University of Oulu (V).

2.2 Experimental design

2.2.1 Phenological observations and natural reproductive success in *Vaccinium*

Flowering phenology and natural reproductive success were monitored as background information (I). The flowering phenology of *V. myrtillus* was monitored in 1993, 1996, 1998 and 2000. The number of buds, open flowers and wilted flowers was counted every few days from small plots sized 25 x 50 cm in 1993 and 50 x 50 cm in the other years. Each time the score was counted, several hundred flowers were checked for their phenological stage. To be able to compare the curves from the different years, I defined the onset of peak flowering as the day when 40 % of the flowers were open, and extrapolated the date from graphical presentations of flower numbers. The onset of peak flowering was related to the accumulated temperature sum (> 5 °C, Growing Degree Day, GDD).

The flowering phenology of *V. vitis-idaea* was monitored in 2000 from 10 permanent plots sized 50 x 50 cm.

Natural reproductive success was assessed as a fruit set percentage [(fruit/flower)*100] and as the number of seeds per berry. Proper seeds, abandoned seeds, and unfertilized ovules were removed from the berries by hand and counted under a dissecting microscope against millimeter paper. Mature seeds were 1 mm in size and coloured brown, while abandoned seeds were of the same size and colour but flat, or smaller than 1 mm and roundish. Small white structures of 1/3 of a millimeter were considered unfertilized ovules. In *V. myrtillus*, fruit set was assessed from the phenology plots in 1993, and from 20 permanent plots in 1996 through 2000. In *V. vitis-idaea*, 10 permanent plots sized 70 x 70 cm were established in 1994, and flower number and berry number were counted from 1994 to 1998.

2.2.2 Manipulating fruiting effort in Vaccinium

In 1998, flowering *V. vitis-idaea* and flowering *V. myrtillus* ramets were chosen and individually marked at natural forest sites in the Oulanka National Park (II). *V. vitis-idaea* was investigated in two slightly different pine-dominated moderately dry heath forests and *V. myrtillus* in spruce-dominated mesic heath forests. For each of the species, fifty ramets were selected at each of six different locations (blocks) within the forests. In total, 300 *V. vitis-idaea* and 300 *V. myrtillus* ramets were chosen for the experiment. Half of the ramets at each location were randomly subjected to a deblossoming treatment (debl) and the other half to a pollination treatment (poll) in 1998 and in 1999. In the deblossoming treatment, flower buds were removed from the ramets. The pollination treatment was conducted in order to encourage ramets to produce berries, and therefore, flowers in each ramet were pollinated with pollen that stemmed from ramets a few meters away from the recipient ramet (cross-pollination). The flowers were open to natural pollination all the time. The number of flowers per ramet was counted prior to deblossoming. In 2000, all the flowering ramets in the experiment were hand pollinated. In all three years, the number of flowers per ramet, flowering frequency, and the number of berries per ramet in the reproducing ramets were recorded. The number of new current-year shoots was counted and shoot length was measured with a ruler (± 0.5 mm). All current-year shoots were measured in *V. vitis-idaea* and a sample of 5 shoots per *V. myrtillus* ramet in the field in 1998 and 1999. In 2000, when fruiting was

completed, the ramets were harvested from the field. Current-year shoots were counted and their lengths were measured from all the current-year shoots.

2.2.3 Conducting hand pollination experiments with *Vaccinium* and *Campanula*

Controlled hand pollinations with *Vaccinium* comprised cross-pollinations at different flower ages (III) and at variable distances between pollen recipient and pollen donor (IV), self-pollinations (III, IV), cross-pollinations (III), and pollen chase experiments (III). In the chase experiment, the experimental flower was first pollinated with self-pollen and then with cross-pollen. Stigma receptivity was assessed by cross-pollinating flowers of different ages. Self-pollinations were conducted to study whether the species were self-compatible, and the pollen chase experiment was conducted to investigate whether the self-pollen fertilized ovules. The reproductive success upon cross-pollination at variable distances between pollen donor and recipient was tested for distance-related genetic population structure. In order to check the capability of *Vaccinium* species to self-pollinate autonomously (autogamy), flowers were not pollinated. The fruit and seed data results from paper (IV) were used to calculate autofertility and self-fertility in *V. myrtillus* and *V. vitis-idaea*.

In hand pollination experiments in the field, individual *Vaccinium* ramets carrying flower buds were covered prior to pollinations with a construction made from metal wire and nylon netting to exclude possible pollinators from the flowers and thus prevent contamination of the flowers with undesired pollen. The nylon bags could be opened and reclosed to make handling of the flowers possible. Flower buds were individually marked, checked every second day or daily, and the day of anthesis (*i.e.* flower bud opening) was recorded. Self-pollinations were conducted by first shaking pollen from the same flower or another flower from the same caged ramet onto a glass microscope slide, and by then touching the stigma with the glass (Molau *et al.* 1989). Pollen can be seen on the microscope slide by naked eye. It was checked with a magnifying glass to determine whether the stigma was covered with pollen. In cross-pollinations, pollen was shaken from usually several flowers that grew at a distance from the recipient ramet onto the glass microscope slide. When applying pollen from several flowers, we evenly spread the pollen from one flower after the other onto the glass. The stigma was then touched with pollen from different places across the slide.

Campanula plants were grown in an insect-free greenhouse (V). Flower buds were marked individually as soon as they developed. Pollinations were conducted when the female receptive phase had started, by rubbing the three stigma lobes of the recipient flower against the stylus of the donor flower. Capsules change colour from green to brown when ripe. Seeds are released through holes that form automatically close to the bottom of the capsule. Capsules were collected shortly before seeds were released and stored in paper bags, and the seeds were counted under a dissecting microscope. Self-pollinations and different sets of cross-pollinations were conducted.

2.2.4 Greenhouse experiment employing mycorrhizal inoculation in *Campanula*

In study (V), *C. rotundifolia* plants were germinated from seed bulk material collected from a subarctic meadow at Kilpisjärvi (69°01'N, 20°50'E; 7679:253, 600 m above sea level). The plants were grown under greenhouse conditions in the non-mycorrhizal stage for 1 year. Three fungal species of northern origin (*Glomus hoi*, *G. claroideum* and *Glomus* sp. isolate) were used to inoculate *Campanula*. Spores of the fungi were propagated in symbiosis with *Sibbaldia procumbens* under laboratory conditions. At the age of 1 year, the *Campanula* plants were planted into soil that consisted of heat-sterilized sand and perlite (9:1) and contained organic fertilizer. Half of the plant material ($n = 49$) was given a solution containing spores of the three arbuscular fungi, and the other half of the plants ($n = 52$) was given an identical solution, but without spores. Hand pollinations were started when flowering commenced two months after the inoculation. At this stage the plants were 14 months old. The phosphorus content of the *C. rotundifolia* plant aboveground biomass was measured by spectrophotometer after HCl extraction of ashed samples. The analyses were performed by Novalab Oy, Karkkila, Finland. Seed phosphorus concentration was measured by plasma emission spectrometry after wet combustion in perchloric acid (HClO₄) – nitric acid (HNO₃) and dilution in HCl. The analysis was performed by Oy Hortilab Ab, Närpiö, Finland (V).

Seeds from the hand pollination treatments were sown in pots with heat-sterilized sand under non-mycorrhizal conditions. The pots were stratified for one month and transferred to a greenhouse with natural light levels and watered with tap water. Ingestad nutrient solution (Ingestad 1979) was applied on six occasions. The emergence of seedlings was recorded daily for two weeks and

every second day thereafter. Relative growth rate (RGR) was calculated from the mean seedling weight at 5 days and 36 days of age as determined from a sample of seedlings per pot.

2.2.5 Statistical analyses

Differences between treatment means were analyzed in paired-samples *t* test, independent samples *t* test, 1-way ANOVA, ANCOVA, two-way factorial ANOVA or repeated-measures ANOVA. Repeated-measures ANOVAs were computed when variables were monitored during consecutive years. The variables were $\lg(x + 1)$, square-root $(x + 0.5)$ or arcsin square-root $(x/100)$ transformed when necessary to meet normality and homoscedasticity. When data could not be normalized, rank-transformations were applied to the data or a non-parametric Mann-Whitney *U* test was conducted. Frequency data were tested using *G* test of independence, Fisher's exact test and χ^2 -test of independence. Statistical analyses were performed employing the SPSS for Windows (SPSS Inc., Chicago, Ill., 8.0, 10.1.4) computer software program.

2.3 Autofertility and self-fertility in Ericaceae

In appendix 1, I present a short review of publications that report reproductive success in controlled hand pollinations in wild ericaceous species. In particular, autofertility and self-fertility were studied. The autofertility index is the ratio of [reproductive success upon autonomous autogamy / reproductive success upon hand cross-pollinations], and the self-fertility index is the ratio of [reproductive success upon hand self-pollinations / reproductive success upon hand cross-pollinations] (compare Lloyd & Schoen 1992). The publications were chosen from the library databases of BIOSIS Previews®. Autofertility and self-fertility values are quoted when given in the reviewed publications or they were calculated from the reported fruit set (percentage of flowers maturing into fruits), seed set (percentage of ovules maturing into seeds) or the number of seeds per fruit. To avoid imprecision, fruit and seed data were only used when given as numerals in the publication, i.e data sets from graphical presentations were not considered. Autofertility and self-fertility were also calculated for *V. myrtillus* and *V. vitis-idaea* from the hand pollination experiment (IV), and included in the further contemplations. The autofertility and self-fertility values were categorized into four arbitrary classes covering the values as follows: 0–0.25; 0.26–0.5; 0.51–

0.75; and > 0.76 . Thereafter, a frequency distribution was created. When a publication reported values for multiple-year or multiple-site observations, mean values were calculated for autofertility and self-fertility for that particular report. I also calculated the mean value when there was more than one publication on the same species. A representation on all the individual records (not shown) gave basically the same pattern as did the mean values.

3 Results and Discussion

3.1 Natural reproductive success in *Vaccinium*

V. myrtillus and *V. vitis-idaea* had a short but intensive flowering period with 10 to 12 days of peak flowering and up to 75 % to 80 % of open flowers presented at the same time (I). Thus, the present *V. myrtillus* and *V. vitis-idaea* populations showed a much higher synchronization of flowering than reported by Jacquemart (1993) for *Vaccinium* populations on a peaty heath in the High Ardennes, where less than 20 % of the flowers were considered open at any time. Highly synchronized flowering among inflorescences has also been reported for a number of Canadian boreal forest herbs (Helenurm & Barrett 1987). The duration of flowering was shorter in the northern boreal *Vaccinium* populations than in ericaceous species at more southern latitudes (Pojar 1974, Jacquemart 1993). Assumedly, the duration and synchrony of flowering reflect the length of the vegetation period. In the present investigation, the onset of peak flowering was associated with the temperature sum in *V. myrtillus*, and this result was congruous between years. A strong correlation of flowering onset with degree days during the weeks preceding flowering has been recorded for *e.g.* arctic plants (Thórhallsdóttir 1998).

The number of flowers and berries produced per plot varied significantly between years in both investigated species, while fruit set and the number of matured seeds per berry did not. In conditions of natural pollination, *V. myrtillus* matured more seeds per berry than *V. vitis-idaea* (23 seeds and 9 seeds, respectively, averaged over two and three years), whereas the mean fruit set varied between 15 % and 27 % in *V. myrtillus* and between 44 % and 57 % in *V. vitis-idaea* (Table 2). Thus, in the monitored forest populations of *V. myrtillus*, fruit set was lower than reported in a clear-cut area (Viramo 1978) and in southern boreal forest populations where fruit sets averaged 60 % (Nousiainen 1980). Differences in fruit set between sites might be based on different resource conditions in the investigated forests and also on differences in pollinator abundance.

Several theories have been formulated to explain why plants produce more flowers than they mature into fruits. The issue of pollen limitation will be briefly discussed in chapter 3.4. The pollinator attraction hypothesis states that plants produce surplus flowers in order to attract more pollinators (Sutherland & Delph

1984). It has indeed been shown that flower density can have positive effects on reproductive success (Bosch & Waser 2001, Knight 2003). However, in the *V. myrtillus* investigated here, neither was there any relationship between fruit set and flower density per plot, nor was fruit set higher in years when plenty of flowers were produced. Nevertheless, significantly positive correlations between the number of flowers and the number of berries per plot were found in three out of five observation years. Also, the number of flowers in individual plots was positively correlated between years in three out of four comparisons. Apparently, some plots had higher fecundity, maybe as a result of locally favourable resource conditions, or maybe because of individual differences between genotypes. During the first three years of monitoring, the mean number of flowers decreased continually from year to year in both species, and so did the number of berries. This fluctuation could be a consequence of possible costs of reproduction to future flowering. Nevertheless, flower production, pollination success and subsequent fruit and seed maturation in general are affected by climatic conditions in a number of species, and fluctuations in seed production in *V. myrtillus* have been associated with climatic conditions, too (Selås 2000).

Table 2. Results summarized from the experiments with *Vaccinium myrtillus* and *Vaccinium vitis-idaea*. ns = statistically non-significant ($P > 0.05$).

Investigated traits	<i>Vaccinium myrtillus</i>	<i>Vaccinium vitis-idaea</i>
Natural reproductive success (I)		
Fruit set	15 % – 27 %, variation ns	44 % – 57 %, variation ns
Number of seeds per berry	22 – 26, variation ns	7 – 12, variation ns
Cost of reproduction (II)		
Survival	no effect	no effect
Frequency of future flowering	reduced, ns	reduced
Future number of flowers	no effect or reduced	reduced, ns
Future growth	no effect or reduced branching	reduced total growth
Mating system (III, IV)		
Autofertility	~zero	zero
Self-fertility (forest populations)	0.62 (fruit data); 0.28 (seed data)	0.75 (fruit data); 0.28 (seed data)
Self-fertility (experimental field)	0.8 (fruit data); 0.2 (seed data)	
Inbreeding depression (experimental field) (III)	$\delta = 0.8$	
Population structure (IV)		
Reproductive success by inter-parent distance	increasing with increasing distance	increasing with increasing distance
Pollinator behaviour (I, IV)		
<i>Bombus</i> sp. flower visitation frequency		~0.2 visits / flower / hr
<i>Bombus</i> sp. flight distances	90 % less than 1 m	90 % less than 1 m

3.2 Cost of reproduction in *Vaccinium*

In both *V. vitis-idaea* and *V. myrtillus*, a cost of reproduction to future flowering and to future growth was found, but not to survival nor to future fruiting success (II; Table 2). The responses of *V. vitis-idaea* and *V. myrtillus* to manipulation of fruiting differed in some respects. Due to an almost complete lack of flowering in *V. vitis-idaea* in the second year, investigating the cost of fruiting to future fecundity and growth was based on one season of fruiting. Even then, an effect of pollination and deblossoming on the frequency of reflowering in *V. vitis-idaea* was seen as late as two years after the initial treatments had been applied, while in *V. myrtillus*, flowering frequency was slightly affected after one year and not at all after two years of treatments. However, *V. myrtillus* responded to previous fruiting with reduced flower production. Common to both species was a positive rather than negative relationship between reproduction and growth. As seen elsewhere, flowering can be positively correlated between years as a function of plant size (Lord 1998, Ehrlén & van Groenendael 2001). Also, survival was not affected in either investigated *Vaccinium* species. The allocation of resources to survival is a prerequisite for future reproduction in perennial plants, and therefore, survival might have priority over reproduction in response to stress (cf. Shefferson *et al.* 2006).

In 1999, one year after the initial treatments were applied, almost none of the *V. vitis-idaea* ramets produced flowers in either of the experimental groups, and total new growth was significantly higher in previously deblossomed ramets. Two years after the initial treatments, previously fruiting ramets reflowered at a lower frequency and produced non-significantly fewer flowers than the deblossomed ramets did, but the treatment groups did not differ in total new growth. Nevertheless, when the flowers in both treatment groups were hand pollinated in 2000, the final year of the experiment, the proportion of ramets setting fruit and the number of berries matured per fruiting ramet did not differ between treatment groups. Fruiting had a positive effect on mean shoot length in 1998, although total new growth per ramet did not differ between pollinated and deblossomed ramets. The same percentage of ramets produced new shoots in both treatment groups in each of the three monitored years.

Unlike the experiment with *V. vitis-idaea*, the pollination and deblossoming treatments could be applied to the ramets of *V. myrtillus* during two subsequent years. In ramets that were pollinated in 1998, the frequency of reflowering was marginally reduced in 1999, and the mean number of flowers in flowering ramets

was significantly reduced in both 1999 and 2000. When all the ramets of the pollination and deblossoming treatments were pollinated in 2000, there was no difference noticed in either the proportion of ramets setting fruit or the mean number of berries per fruiting ramet. However, one fifth of the ramets in the pollination treatment had not matured berries in 1999. Since this behaviour could have been a cost response, these ramets were investigated as a separate group (pollinated, no berry 1999: poll-NB99). Indeed in 2000, the mean number of flowers and the mean number of berries were smaller in the poll-NB99 ramets than in the other pollinated or deblossomed ramets. Also, the mean number of current-year shoots in the poll-NB99 ramets was reduced in both 1999 and 2000. However, the total current-year growth remained unaffected and thus, the cost of reproduction to vegetative traits was expressed as a modification of ramet architecture.

While a trade-off between reproduction and growth could have been expected within the same season, *V. myrtillus* showed no trade-offs and the *V. vitis-idaea* ramets showed the opposite in terms of mean shoot length in the first year. If a reproducing ramet is able to increase its sink strength and/or assimilation (Reekie & Bazzaz 1987), it might be able to maintain both reproduction and growth, or as seen here in *V. vitis-idaea*, even increase growth. Previously, some positive effects of reproduction on growth have been observed in e.g. *Plantago rugelii* (Reekie & Bazzaz 1992) and *Primula veris* (Lehtilä & Syrjänen 1995). Also, greater translocation of resources from below-ground storages or clonal integration may become important in compensating increased demands in ramets recovering from damage (Pitelka & Ashmun 1985, Tolvanen 1994) or in reproducing ramets (Ganger 1997). When the pollination level was experimentally increased in clonal *Maianthemum canadense*, severing rhizomes had a limiting effect on the number of fruits, seeds and seeds/fruit matured, but under natural levels of pollination, rhizome severing had no effect on reproductive success as compared with plants whose rhizomes were left intact (Ganger 1997).

Plant responses to manipulation of reproductive costs potentially vary with habitat (Syrjänen & Lehtilä 1993, Hemborg 1998), thus possibly reflecting environmental effects on responses. Also individual *V. myrtillus* ramets might have experienced micro-sites that were heterogeneous in terms of resources, and the poll-NB99 ramets possibly grew in poorer conditions. Furthermore, if it is assumed that the poll-NB99 ramets in *V. myrtillus* did not develop enough sink strength to compete with sister ramets within the same clone for storage resources, plasticity in clonal integration might explain why some ramets showed

costs while others did not (see Sachs & Novoplansky 1997, Hellström *et al.* 2006). As a result, reproductive effort was reduced and ramet architecture altered in the poll-NB99 ramets of *V. myrtillus*, but still, total growth in these ramets did not differ from that of the other pollinated or deblossomed ramets. Suppressing berry maturation and reducing branching may have helped the poll-NB99 ramets to overcome a temporary resource shortage. Changed branching patterns in response to low resource conditions has been predicted and observed in herbaceous plants (Benner 1988, Aarssen & Irwin 1991, Bonser & Aarssen 1996, Rautio *et al.* 2005), and it has also been noticed in ericaceous species (Salemaa & Sievänen 2002, Zvereva & Kozlov 2005).

Literature reports costs of reproduction to future reproduction (Ågren 1988, Hemborg 1998, Houle 2001, Turcotte & Houle 2001), future growth (Wyka & Galen 2000, Ehrlén & van Groenendael 2001) and survival (Ågren 1988), while some studies were not able to find any cost of reproduction (Lord 1998, García & Ehrlén 2002). So far, there are few publications addressing the cost of reproduction in ericaceous species. In *Empetrum nigrum* ssp. *hermaphroditum*, flower bud removal in one season was followed by increased flower bud production the next year (Mutikainen & Ojala 1993), thus basically indicating an expression of costs. But, in the study by Mutikainen and Ojala (1993), increased flower bud production was accompanied by reduced growth and branching, and might therefore indicate trade-offs within the same year rather than positive effects of prevented reproduction to future fecundity. Furthermore, a study by Elle (1996) showed that allocation strategies can differ between individual genotypes in *Vaccinium macrocarpon*. Theoretically, our finding that some *V. myrtillus* ramets expressed costs, while others did not, might also be explained by differences between genotypes.

In *V. vitis-idaea* ramets, the almost complete absence of flowering one year after the initial treatments were applied might have been a cost response to flower production as such, or it was caused by morphological constraints. The formation of inflorescences terminates growth of the main ramet or branching shoot in *V. vitis-idaea*, and ramet growth has to continue by shoot production from axillary buds. In *V. myrtillus*, flower buds and new shoots are produced at the same time. In fact, growth habit in concordance with meristem limitations could be the reason for the different and stronger costs expressed in *V. vitis-idaea* compared with *V. myrtillus*. In a previous study with *V. myrtillus* and *V. vitis-idaea*, no costs of reproduction were seen in a number of vegetative traits, but sugar and starch levels in *V. vitis-idaea* were slightly affected in deblossomed ramets as compared

with flowering ramets (Tolvanen & Laine 1997). Moreover, in *V. vitis-idaea* in that same study, costs to vegetative traits were detected in flowering and deblossomed ramets as compared with non-flowering ramets. Altogether, these results might indicate a stronger disposition in *V. vitis-idaea* to express costs than in *V. myrtillus*, perhaps as a consequence of the species' growth form. In general, deciduous species have higher photosynthetic and growth rates and thus are able to *e.g.* recover faster from herbivory (Tolvanen 1994).

3.3 Reproductive success in hand pollinated *Vaccinium* and *Campanula*

In both *Vaccinium* species and *C. rotundifolia*, seed production was reduced upon self-pollination in comparison with cross-pollination. This seemed to be due to early-acting inbreeding depression, at least in *V. myrtillus* (III). The lower seed set in hand self-pollinated than in hand cross-pollinated *C. rotundifolia* flowers was probably caused by partial self-incompatibility (V). Nevertheless, *C. rotundifolia* also suffered from inbreeding depression, which was expressed during seed germination (V).

V. myrtillus flowers were capable of producing seeds in hand pollinations up to an age of 7 days, and flower age had no effect on the number of seeds matured in either cross-pollinations or self-pollinations (III). Wood (1962) reported *V. angustifolium* flowers to be capable of setting fruit up to an age of 7 days, also. In the pollen chase experiment with *V. myrtillus*, cross-pollen applied 1 day, 2 days, or 3 days after self-pollen did not increase the number of seeds beyond the number of seeds matured in hand self-pollinations only (III). Furthermore, the number of aborted seeds in the chase treatment was equal to those in the self-pollinations, and consequently, self-pollen must have fertilized ovules, some of which were then aborted. These results are in general concordance with an earlier study employing pollen chase experiments with *V. corymbosum* (Krebs & Hancock 1990). In both *V. myrtillus* and *V. vitis-idaea*, seed production was reduced and the number of aborted seeds per fruit was increased in self-pollinated as compared with cross-pollinated berries (III, IV). When self-pollen and cross-pollen was applied simultaneously to *V. myrtillus* flowers, the number of seeds and the number of aborted seeds per berry ranged between the number of seeds and the number of aborted seeds in self-pollinations and cross-pollinations. Similar observations have been made in pollinations with pollen mixes in

Vaccinium corymbosum (Vander Kloet & Lyrene 1987), *Calluna vulgaris* (Mahy & Jacquemart 1999), and *V. myrtillus* in central Europe (Raspé *et al.* 2004).

In the next step, the results of the different pollination treatments were used to develop a regression model that evaluated the occurrence of inbreeding depression versus self-incompatibility from the numbers of matured and aborted seeds per fruit (III). From this model it was concluded that *V. myrtillus* suffered from inbreeding depression at seed maturation, since the number of mature seeds and aborted seeds following hand self-pollinations and hand cross-pollinations had a significant negative correlation.

Neither of the *Vaccinium* species appeared to be effectively autogamous (III, IV), since the flowers generally did not develop into fruits when pollinators were excluded. Also, there was no indication of apomixis as tested in *V. myrtillus* (III). Autofertility was zero in *V. vitis-idaea* and close to zero in *V. myrtillus* when calculated from fruit set at the forest sites (IV, Appendix 1). In both species, self-fertility was higher in terms of fruit set than in terms of seeds matured per berry. Self-fertility in *V. vitis-idaea* was 0.75 and 0.28 as calculated from fruits and seeds, respectively. In *V. myrtillus*, self-fertility was 0.6 based on fruit set, and 0.24 based on seed number, averaged for the two forest sites (IV, Appendix 1).

The self-pollination and cross-pollination experiment with *V. myrtillus* in the experimental field showed that there appeared to be variation in self-fertility between clones (III). In thirteen out of eighteen clones, self-fertility was less than or equal to 0.25, in four clones the value was between 0.28 and 0.4, and in one clone it was 0.74 (compare relative survivorship R in Table 2 in paper III). The mean of these values was 0.2 ± 0.04 (\pm SE, $n = 18$), and hence comparable to the self-fertility values gained from the experiments at the forest sites that employed separate ramets for self-pollinations and cross-pollinations (IV). Using the individual self-fertility values, or relative survivorship estimates, a mean inbreeding depression of $\delta = 0.8 \pm 0.04$ (\pm SE) was calculated for the *V. myrtillus* clones at the experimental field. The clones had an average of 7.8 ± 0.8 ($n = 18$) lethal equivalents per zygote.

Equally high estimates of lethal equivalents have been reported for coniferous trees (Sorensen 1969, Kärkkäinen *et al.* 1996) and other long-lived perennial species (Seavey & Carter 1994), while lower values of one to two lethal equivalents per zygote have been calculated for some short-lived herbaceous species (Levin 1984, Lynch & Walsh 1998). Within the Ericaceae, inbreeding depression at seed maturation has been recognized to be the reason for reduced self-fertility in *e.g.* *Calluna vulgaris* (Mahy & Jacquemart 1999), *Kalmia latifolia*

(Rathcke & Real 1993), *V. angustifolium* and *V. myrtilloides* (Hokanson & Hancock 2000), *V. corymbosum* (Krebs & Hancock 1990, 1991), and *V. myrtillus* and *V. vitis-idaea* (Guillaume & Jacquemart 1999). The studies that have estimated inbreeding depression show rather high levels (Rathcke & Real 1993, Guillaume & Jacquemart 1999, Mahy & Jacquemart 1999).

In a number of different organisms, the expression of inbreeding depression has been shown to be greater in stressful environments than in benign environments (Dudash 1990, Armbruster & Reed 2005). Kärkkäinen *et al.* (1996) observed less inbreeding depression in northern populations than in southern populations of *Pinus sylvestris* in Finland, which they explained with more frequent self-fertilization and assumedly stronger selection against deleterious alleles in the northern populations. Nevertheless, no difference was noticed in inbreeding depression as calculated for *V. myrtillus* in the northern boreal forest population (III) as compared with a population in central Europe (Guillaume & Jacquemart 1999).

Isozyme studies have revealed intermediate outcrossing rates in *V. myrtillus* in central Europe with an estimated selfing rate of 28 % (Jacquemart *et al.* 1994). Even though selfed seeds are most probably also matured in the boreal *Vaccinium* populations, there appears to be little recruitment of new seedlings in undisturbed sites (Ericson 1977, Eriksson & Fröborg 1996, Hautala *et al.* 2001), assumedly because germination in general is obstructed by dense understorey and ground vegetation. If there is little establishment of selfed individuals that reach maturity, removal of deleterious recessive alleles may be slow albeit selfing.

In the experimental hand pollinations with *C. rotundifolia* (V), pollination treatment, whether self-pollen or cross-pollen was applied to the flowers, had a significant effect on the number of seeds and the number of fertilized ovules per capsule. Since both the number of fertilized ovules and mature seeds was lower in self-pollinations than in cross-pollinations, it was concluded that seed set in *C. rotundifolia* is most probably affected by a self-incompatibility system. If there was no self-incompatibility system at all, the number of fertilized ovules in self-pollinated flowers should not deviate from that in cross-pollinated flowers. Also, the pollination type affected fruit set percentage. More cross-pollinated flowers set fruit than unpollinated flowers did in either non-mycorrhizal or mycorrhizal plants or self-pollinated flowers in the non-mycorrhizal plants. The fruit set percentage in self-pollinated mycorrhizal plants did not differ from that in any of the other pollination treatments. Self-pollinated flowers in non-mycorrhizal plants and unpollinated flowers in both non-mycorrhizal and mycorrhizal plants matured

significantly fewer fruits than cross-pollinated flowers did. Unpollinated flowers spontaneously set fruit, but with 68 % and 46% fewer seeds per capsule than in self-pollinated flowers in non-mycorrhizal and mycorrhizal plants, respectively. Seeds from self-pollinations had a lower germination percentage than seeds from cross-pollinations, probably as a result of inbreeding depression acting during germination.

The expression of self-incompatibility has been noticed to vary in some plant species in dependence on variable environment (Becerra & Lloyd 1992, Reinartz & Les 1994). In some other species, self-incompatibility can lose its strength with increasing flower age (Vogler *et al.* 1998, Goodwillie *et al.* 2004, Travers *et al.* 2004). In *Campanula rapunculoides* for instance, an age-dependent breakdown of the gametophytic self-incompatibility system has been identified and moreover genotypic variation between individual plants in the strength of self-incompatibility (Vogler *et al.* 1998). In the presently investigated *C. rotundifolia* material from subarctic Finnish Lapland, there was no effect of mycorrhiza on the ability to mature seeds upon self-pollination, but fruit set success in mycorrhizal plants was not different from fruit set of any of the cross-pollinations, while it was different in non-mycorrhizal plants. This result seems to indicate a slight improvement in fruit set upon experimental self-pollination in mycorrhizal plants as compared with non-mycorrhizal plants. *C. rotundifolia* from southern Sweden has been referred to as self-incompatible (Nyman 1993) and non-autogamous (Nyman 1992). Under experimental greenhouse conditions, the *C. rotundifolia* plants from seed material from subarctic Finland were capable of some autonomous self-pollinations.

3.4 Plant reproduction in response to plant-pollinator interaction

Low natural fruit set could be a result of pollen limitation (Bierzychudek 1981, Burd 1994), and this might be the case particularly with *V. myrtillus*. In *V. vitis-idaea*, the mean visitation frequency by bumblebees (*Bombus* sp.) to individual flowers was calculated as 0.1975 (\pm 0.1572, SD) visits / flower / hr, meaning that theoretically each flower got visited by a bumblebee every five hours (I). This visitation frequency is of the same magnitude as reported for *Vaccinium* species elsewhere (Jacquemart 1993) and for *e.g.* perennial plants in boreal (Totland *et al.* 2006) and alpine ecosystems (Bingham & Orthner 1998). Of the observed pollinators during peak flowering, 86 % belonged to the genus *Bombus*, making bumblebees the most frequent visitors during the observation periods. Moreover,

the pollinator observation plot reached a fruit set of 73 %, and thus indicated at least successful pollination. However, the recorded visitation frequency has to be considered with care, since observations were both restricted in time and space. In the hand pollination experiments with both *V. myrtillus* and *V. vitis-idaea* (III, IV), there was hardly any sign of autonomous self-pollinations, implying that flowers need to be pollinated in order to set fruit. However, higher autofertility as calculated from fruit set has been reported for *V. myrtillus* by Jacquemart & Thompson (1996).

The distance between pollen donor and pollen recipient had a significant effect on fruit set in one out of two investigated *V. myrtillus* populations (IV). Fruit sets were lower in the naturally (open) pollinated and self-pollinated ramets than in the inter-population cross-pollinations, and fruit set in self-pollinations was also lower than in cross-pollinations at a 10 m distance. In *V. vitis-idaea*, open pollinated flowers had as high fruit set as hand pollinated flowers did. Species or populations have been considered pollen limited when experimental pollinations can increase reproductive success in comparison with natural pollination (Burd 1994). Based on this assumption, the results suggest that natural fruit set in *V. myrtillus* might be subject to pollen limitation, while this does not appear to be the case in *V. vitis-idaea*. However, the present experiment was not designed to test this issue in particular. Moreover, the number of seeds in the naturally pollinated *V. myrtillus* ramets equalled the number of seeds in self-pollinations, and differed from all the cross-pollinations at increasing distance from the pollen recipient. In *V. vitis-idaea*, the number of seeds per berry in natural pollinations was intermediate between self-pollinations and cross-pollinations, without differing significantly from either of them. Therefore, it seems that seed production in both species could be pollen-limited, too, or more specifically, assumedly limited by the availability of cross-pollen. As demonstrated in the pollen chase experiment (III), when cross-pollen arrived one day after self-pollen, the number of seeds equalled the number of seeds in pure self-pollinations. Pollen limitation experiments that apply supplemental hand pollination to naturally pollinated flowers could easily underestimate the effect if the plant species is self-compatible but suffers from high inbreeding depression at seed maturation. In this case, the number of seeds matured might depend on whether flowers have already received self-pollen. Thus, pollen-limitation experiments might remain without any effect on the number of seeds.

The number of seeds matured per fruit increased with increasing distance between pollen donor and pollen recipient in *V. myrtillus* and to some degree also

in *V. vitis-idaea* (IV). Pollination treatment also had a strong effect on the number of aborted seeds per berry in both *Vaccinium* species, as it decreased with increasing distance between mating partners. Reproductive success in the inter-populational cross-pollinations in *V. myrtillus* did not give evidence of outcrossing depression (but see Price & Waser 1979, Waser & Price 1989, 1991, Schemske & Pautler 1984). However, negative effects might not become visible in seed production but in offspring traits (Waser & Price 1989).

A number of studies have addressed the effect of distance between mates on reproductive success. While some studies failed to establish any relationship (Newport 1989, Fenster 1991, Schlichting & Devlin 1992), others reported improved reproductive success with increasing distance between pollination partners (Levin 1984, 1989, Dudash 1990). In *Agave schottii*, a long-lived clonal plant, seed set was significantly lower in cross-pollinations of 1 m than of 10 m, 100 m or 2500 m distance between pollen donor and pollen recipient (Trame *et al.* 1995). However, despite clonality, the pollen donors from 1 m distance were genetically similar but not identical to the pollen recipient plant, and seed set was explained by inbreeding depression upon biparental crossing over short distances (Trame *et al.* 1995).

The majority of between-flower or between-inflorescence travels by bumblebees were at distances of less than 1 m, an observation that is consistent with the findings on bumblebee foraging behaviour in other studies (Schmitt 1980, Waddington 1983, Totland 1994). At this distance, seed production per berry in hand pollinations was lower than in cross-pollinations, but still higher than in pure self-pollination in *V. myrtillus*, and this might have been at least partly due to uniparental inbreeding in the form of geitonogamy. In the cross-pollinations, pollen was applied as a mixture from five donors. If any of the donor ramets belonged to the same genet as the recipient ramet, the pollen load might have consisted of self-pollen and cross-pollen. Moreover, naturally pollinated flowers matured fewer seeds than hand cross-pollinated flowers did, which could be a result of insect-mediated self-pollination within clones (IV), if one neglects that pollen carry-over takes place between flower or inflorescence visits. Nevertheless, it remains to be investigated how much pollinator-assisted within-clone and/or within-flower self-pollination takes place (see Eckert 2000).

Clone size was not determined in the present study, but excavations of *V. myrtillus* clones from forests in Sweden have shown that rhizomes may grow up to 5.5 m in length (Flower-Ellis 1978). Furthermore, Albert *et al.* (2003) employed molecular markers in a Belgian population and showed that *V. myrtillus*

clones were on average 2 m in size, with the biggest clone being 5.5 m in size. These clone sizes could also be projected by the results from the present pollination experiments.

3.5 Plant reproduction in response to plant-fungus interaction

In the perennial herb *C. rotundifolia*, mycorrhiza had both positive and negative effects on the parental generation, while at the same time having positive effects on the offspring generation (V). Non-mycorrhizal mother plants had a 33 % higher total biomass, but a 15 % lower root-shoot ratio than mycorrhizal plants. Moreover, non-mycorrhizal plants produced more flowers per plant than mycorrhizal plants did (15.8 ± 0.9 and 9.5 ± 0.6 , respectively; mean \pm SE). Mycorrhiza had no effect on the number of seeds matured per capsule, the number of fertilized ovules per capsule or seed weight per seed. Mycorrhizal plants are often larger and have more flowers than non-mycorrhizal plants (Koide *et al.* 1988, Stanley *et al.* 1993, Poulton *et al.* 2002). In the experiment with *C. rotundifolia*, the opposite was noticed. No growth benefit from mycorrhiza may indicate competition for assimilates between the host and the root symbionts, and consequently a high carbon cost of mycorrhizal symbiosis (Buwalda & Goh 1982). Some other studies have also recorded lower biomass production in mycorrhizal plants, which they explained with a high inorganic P supply (Peng *et al.* 1993, Graham *et al.* 1996) or low light conditions (Bethlenfalvay & Pacovsky 1983). As previously shown, *C. rotundifolia* had improved seedling growth in single fungus inoculations with *Glomus claroideum*, but not with two other fungi (Kytöviita *et al.* 2003). Assumedly, plant benefit from mycorrhiza may be different in different plant life stages (Koide 1991).

Positive effects of mycorrhiza on pollen production (Poulton *et al.* 2002, Philip *et al.* 2001), pollen tube growth (Poulton *et al.* 2001) and pollen grain diameter (Lau *et al.* 1995) have been reported for various plant species. Moreover, pollen that was produced in low nutrient conditions was inferior in siring seeds under competitive conditions (Young & Stanton 1990, Lau & Stephenson 1993). If improved pollen traits, such as size and particularly pollen tube growth, translate into higher siring abilities, then mycorrhiza could have an effect on male reproductive success. As was seen in Young & Stanton (1990), siring ability was different only when pollen from low-nutrient plants was applied together with pollen from control plants. In *C. rotundifolia*, mycorrhiza had no

effect on the siring ability of pollen in hand pollinations where, however, single donor pollen loads were applied.

Mycorrhizal *C. rotundifolia* plants had a higher shoot phosphorus concentration and content than non-mycorrhizal plants, and the mycorrhizal plants also matured seeds with a higher phosphorus concentration. This result is concordant with other studies showing that the mycorrhizal plants rich in phosphorus are able to also produce seeds rich in phosphorus (Bryla & Koide 1990, Lu & Koide 1994, Stanley *et al.* 1993). It is recognized that maternal environmental conditions can affect offspring quantity and quality (Roach & Wulff 1987, Parrish & Bazzaz 1985, Sultan 1996, Cheplick & Sung 1998).

Despite the higher phosphorus concentration, seeds germinated equally fast and well irrespective of the fungal treatment applied to the mother plant in *C. rotundifolia*. In some studies, no effects on seed germination of improved nutrient levels in maternal plants have been observed (Heppell *et al.* 1998, Parrish & Bazzaz 1985), while positive effects were noticed in others (Cheplick & Sung 1998). The seedlings from mycorrhizal *C. rotundifolia* plants had a higher relative growth rate (RGR) than did seedlings from non-mycorrhizal mother plants. A higher seed phosphorus concentration and higher offspring growth might be related to one another, as positive effects on both measures by mycorrhiza have also been reported in other studies (Lewis & Koide 1990, Heppell *et al.* 1998).

At first glance, it appears that non-mycorrhizal plants have a fitness advantage, as they produced more flowers and therefore potentially more offspring than the mycorrhizal plants did. However, only some flowers were hand pollinated in the present experiment, and it is impossible to predict total plant seed number. When pollination and fruit set are more abundant, the number of seeds might become restricted by resources or possibly traded-off against the number of capsules matured.

Plants with many flowers may be more attractive to insect pollinators. In a few investigations, mycorrhizal plants were more often visited by insect pollinators and had higher seed production than non-mycorrhizal plants did (Gange & Smith 2005, Wolfe *et al.* 2005). Nevertheless, in these studies the more frequent visitation of mycorrhizal plants by insect pollinators was most probably a consequence of mycorrhizal plants having larger inflorescences or larger flowers. Higher pollinator visitation rates should have a positive effect on plant fitness. However, Harder & Barrett (1995) observed that hermaphrodite *Eichhornia paniculata* plants with a large floral display experienced more geitonogamous self-pollination than did plants with a smaller floral display, and

the same was observed for *Campanula americana* (Galloway *et al.* 2002). Theoretically, if geitonogamy increases in natural pollinations in many-flowered *C. rotundifolia* too, higher reproductive effort could possibly be opposed by lower reproductive success upon selfing, or lower quality offspring.

3.6 Autofertility and self-fertility in Ericaceae

A total of 24 species belonging to 9 genera were reviewed for their levels of autofertility and self-fertility (Fig. 1, Appendix 1). Autofertility values based on fruit data ranged between zero and 0.84, and the values based on seed data ranged between zero and 0.92. Most of the reviewed species showed low autofertility, *i.e.* there was no or only some spontaneous fruit and seed set. For nine out of 22 species, there was no spontaneous fruit set reported at all, and for two further species the reported fruit set was zero in one publication and low in another. However, three species showed outstanding autofertility: *Andromeda polifolia* and *Kalmia angustifolia* in terms of fruit set (0.73 and 0.84, respectively), and *Rhododendron ferrugineum* in terms of seeds per fruit (0.92) (Appendix 1). For eleven species, autofertility could be provided from both fruit data and seed data. When these values were plotted against each other by plant species, a significant positive correlation was observed [Spearman's $\rho = 0.755$, p (two-tailed) = 0.007, $n = 11$]. The more a species was able to produce fruits autonomously, the more seeds were produced in these fruits.

Self-fertility ranged from zero to 1.12 when calculated from fruit data and from zero to 0.96 when calculated from seed data (Appendix 1). In most species, seed set was considerably lower after hand self-pollination than after cross-pollination. In only one species, *Rhododendron moulmainense*, fruit set was zero upon hand self-pollination. For *Vaccinium uliginosum*, fruit set was zero in one publication (Fröborg 1996) but much higher in another one (Jacquemart & Thompson 1996). For fifteen species, self-fertility could be calculated from fruit and seed data, and there was no statistically significant correlation between the two (Spearman's $\rho = 0.338$, $p < 0.218$, $n = 15$). Thus, for self-fertility it can not be concluded from fruit data on seed data and vice versa. Self-fertility based on seed data was low for most species, whereas higher self-fertility was observed when the values were calculated from fruit data (Appendix 1).

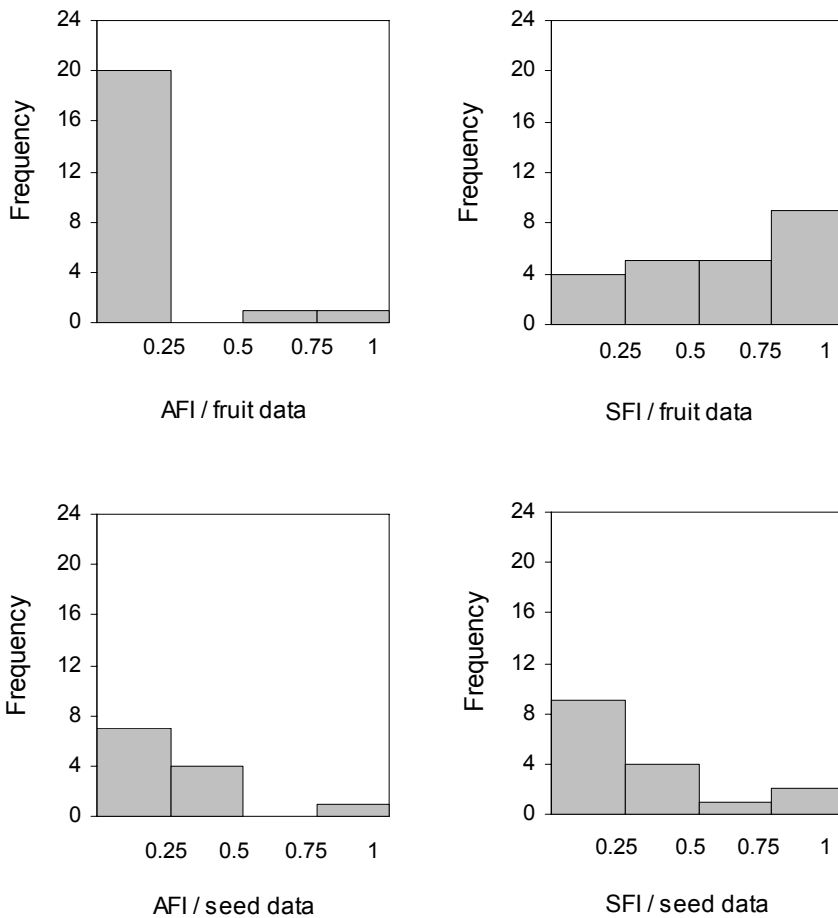


Fig. 1. Frequency distribution of autofertility values (AFI) and self-fertility values (SFI) calculated for ericaceous species on the basis of fruit data and seed data, respectively.

Comparative studies on plant mating systems have shown that related plant taxa often exhibit a wide variety of flower morphologies and they also vary in their ability to self-pollinate and self-fertilize (Schoen *et al.* 1997, Goodwillie 1999). Variation in autofertility and self-fertility was seen between species within the same genera, as in *Andromeda*, *Erica*, and *Rhododendron* (Appendix 1). Also, some variation in autofertility and self-fertility was observed in the same species

investigated at different geographic locations, like *Vaccinium myrtillus*, *V. uliginosum*, *V. vitis-idaea* and *V. oxycoccus* (Reader 1977, Fröborg 1996, Jacquemart & Thompson 1996). Among these, the autofertilities of *V. myrtillus* and *V. vitis-idaea* were the lowest, as reported in the present work (IV), which investigated the northernmost sites. The observed variation in reproductive success upon experimental pollination for the same species at different geographic sites could reflect differences in mating system.

Calculations of self-fertility from fruit data tended to overestimate self-fertility because apparently pollination initiated fruit development even though only some seeds were matured. If a plant's offspring under natural conditions are the product of selfing, it is not necessarily because of high spontaneous self-pollination, but may be pollinator-mediated (Leclerc-Potvin & Ritland 1994; Eckert 2000). In a survey of 66 plant species, Lloyd & Schoen (1992) found a significant positive relationship between self-compatibility and autofertility.

4 Conclusions

In the presently investigated boreal forest sites, natural reproductive success was low in *V. myrtillus*, the earlier flowering species, while *V. vitis-idaea* showed higher fruit set. Flowers set fruit equally well with self-pollen and cross-pollen in most of the hand pollination experiments, and therefore natural fruit set does not seem to be limited by pollen origin, while the number of seeds probably is. It is very likely that fruit set is pollinator limited. *V. vitis-idaea* experienced abundant enough pollinator visitation at peak flowering to ensure that, theoretically, each flower was visited. However, this result is tentative, since observations on pollinators were conducted during one season only.

Cost of reproduction to future fecundity and growth was observed in both *Vaccinium* species, but reproduction appears to be less costly in deciduous *V. myrtillus* than in evergreen *V. vitis-idaea*. Already a single event of reproduction caused costs to subsequent flowering and growth in *V. vitis-idaea*, whereas the majority of ramets in *V. myrtillus* did not show any costs, even though they produced berries two years in a row. As seen in *V. vitis-idaea*, two years after the fruiting, ramets had recovered in terms of vegetative growth, whereas reproductive effort was still negatively affected by previous reproduction. The costs to future fecundity and future branching in one fifth of the *V. myrtillus* ramets had been evoked by intense fruiting upon hand pollination, which was higher than fruit set following natural pollination. Therefore, it remains to be examined whether natural fruit set levels in *V. myrtillus* are capable of causing costs after all. The results also emphasize that it can be important to conduct studies over several years in order to detect responses to reproduction. Fruiting costs to future fecundity in *V. vitis-idaea* would have remained unnoticed in a study lasting one or two years.

In the clonal *Vaccinium* species, plant-pollinator interactions have the potential to nourish self-pollination, because pollinators mostly travelled distances between flowers and between inflorescences at ranges that presumably enable geitonogamous pollination within clones. If pollen carry-over between flower or inflorescence visits is neglected, it can be assumed that in natural pollinations there is a high chance of seeds being matured as a result of self-pollination or pollination between genetically close relatives (biparental inbreeding). In the presently investigated *Vaccinium* species, seed production is potentially constrained by clonal growth in conjunction with pollinator behaviour.

The investigated *V. myrtillus* and *V. vitis-idaea* had no mechanisms for avoiding self-pollination or self-fertilization, and they obviously suffered from inbreeding depression at seed maturation. The level of inbreeding depression at seed maturation in the investigated *V. myrtillus* population was as high as reported for other ericaceous species and another central European *V. myrtillus* population. Within the reviewed Ericaceae, most of the species did not autonomously self-pollinate, and the species that did produced few seeds more often than many seeds. Some species showed a high capability to autonomously self-pollinate. The ability to produce fruit upon self-pollination ranged from weak to good, but the number of seeds matured in self-pollinations was low in most species.

Mycorrhiza inferred costs to the maternal generation of *C. rotundifolia* in terms of reduced growth, and reduced reproductive capacity in terms of flower production. At the same time, symbiosis had advantageous effects on the offspring by improving the seed phosphorus status, which was associated with a higher growth rate during the first weeks of seedling growth. Offspring from mycorrhizal mother plants might have a competitive advantage during seedling establishment. Also, *C. rotundifolia* expressed inbreeding depression during germination, but the reduced number of seeds in self-pollinations as compared with cross-pollinations is likely to be based on self-incompatibility. Although mycorrhiza had no effect on siring ability or seed maturation in hand pollinations, fruit production following self-pollination tended to be better in mycorrhizal plants.

References

- Aarssen LW & Irwin DL (1991) What selection – herbivory or competition? *Oikos* 60: 261-262.
- Ågren J (1988) Between-year variation in flowering and fruit set in frost-prone and frost-sheltered populations of dioecious *Rubus chamaemorus*. *Oecologia* 76: 175-183.
- Albert T, Raspé O & Jacquemart A-L (2003) Clonal structure in *Vaccinium myrtillus* L. revealed by RAPD and AFLP markers. *International Journal of Plant Sciences* 164: 649-655.
- Aparicio A & García-Martín F (1996) The reproductive biology and breeding system of *Erica andevalensis* CABUZEDO & RIVERA (Ericaceae), an endangered edaphic endemic of southwestern Spain. Implications for its conservation. *Flora* 191: 345-351.
- Armbruster P & Reed DH (2005) Inbreeding depression in benign and stressful environments. *Heredity* 95: 235-242.
- Barrett SCH (1988) The evolution, maintenance, and loss of self-incompatibility systems. In: Lovett Doust JL & Lovett Doust LL (eds) *Plant reproductive ecology: patterns and strategies*. Oxford University Press, New York, New York, USA, 98-124.
- Barrett SCH & Eckert CG (1990) Variation and evolution of mating systems in seed plants. In: Kawano S (ed) *Biological approaches and evolutionary trends in plants*. Academic Press, Harcourt Brace Jovanovich, Publishers, London, 229-254.
- Barrett SCH, Harder LD & Worley AC (1996) The comparative biology of pollination and mating in flowering plants. *Philosophical Transactions of the Royal Society of London B*: 351: 1271-1280.
- Becerra JX & Lloyd DG (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.
- Benner BL (1988) Effects of apex removal and nutrient supplementation on branching and seed production in *Thlaspi arvense* (Brassicaceae). *American Journal of Botany* 75: 645-651.
- Bethlenfalvai GJ & Pacovsky RS (1983) Light effects in mycorrhizal soybeans. *Plant Physiology* 73: 969-972.
- Biere A (1995) Genotypic and plastic variation in plant size: effects on fecundity and allocation patterns in *Lychnis flos-cuculi* along a gradient of natural soil fertility. *Journal of Ecology* 83: 629-642.
- Bierzychudek P (1981) Pollinator limitation of plant reproductive effort. *American Naturalist* 117: 838-840.
- Bingham R & Orthner AR (1998) Efficient pollination of alpine plants. *Nature* 391: 238-239.
- Bonsler SP & Aarssen LW (1996) Meristem allocation: a new classification theory for adaptive strategies in herbaceous plants. *Oikos* 77: 347-352.
- Bosch M & Waser NM (2001) Experimental manipulation of plant density and its effect on pollination and reproduction of two confamilial montane herbs. *Oecologia* 126: 76-83.

- Bryla DR & Koide RT (1990) Role of mycorrhizal infection in the growth and reproduction of wild vs. cultivated plants. II. Eight wild accessions and two cultivars of *Lycopersicon esculentum* Mill. *Oecologia* 84: 82-92.
- Burd M (1994) Bateman's principle and plant reproduction; the role of pollen limitation in fruit and seed set. *The Botanical Review* 60: 83-139.
- Buwalda JG & Goh KM (1982) Host-fungus competition for carbon as a cause of growth depression in vesicular-mycorrhizal ryegrass. *Soil Biology and Biochemistry* 14: 103-106.
- Campbell DR & Halama KJ (1993) Resource and pollen limitations to lifetime seed production in a natural plant population. *Ecology* 74: 1043-1051.
- Charlesworth D & Charlesworth B (1987) Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* 18: 237-268.
- Cheplick GP & Sung LY (1998) Effects of maternal nutrient environment and maturation position on seed heteromorphism, germination, and seedling growth in *Triplasis purpurea* (Poaceae). *International Journal of Plant Sciences* 159: 338-350.
- Delph LF, Johannsson MH & Stephenson AG (1997) How environmental factors affect pollen performance: ecological and evolutionary perspectives. *Ecology* 78: 1632-1639.
- Dudash M (1990) Relative fitness of selfed and outcrossed progeny in a self-compatible, protandrous species, *Sabatia angularis* L. (Gentianaceae): a comparison in three environments. *Evolution* 44: 1129-1139.
- Eckert CG (2000) Contributions of autogamy and geitonogamy to self-fertilization in a mass-flowering, clonal plant. *Ecology* 81: 532-542.
- Ehrlén J & van Groenendael J (2001) Storage and the delayed costs of reproduction in the understorey perennial *Lathyrus vernus*. *Journal of Ecology* 89: 237-246.
- Elle E (1996) Reproductive trade-offs in genetically distinct clones of *Vaccinium macrocarpon*, the American cranberry. *Oecologia* 107: 61-70.
- Ericson L (1977) The influence of voles and lemmings on the vegetation in a coniferous forest during a 4-year period in northern Sweden. *Wahlenbergia* 4: 1-114.
- Eriksson O & Fröborg H (1996) "Windows of opportunity" for recruitment in long-lived clonal plants: experimental studies of seedling establishment in *Vaccinium* shrubs. *Canadian Journal of Botany* 74: 1369-1374.
- Escaravage N, Pornon A, Doche B & Till-Bottraud I (1997) Breeding system in an alpine species: *Rhododendron ferrugineum* L. (Ericaceae) in the French northern Alps. *Canadian Journal of Botany* 75: 736-743.
- Fægri K & van der Pijl L (1979) *The principles of pollination ecology*. 3rd ed, Pergamon Press, Oxford, UK.
- Fenster CB (1991) Effect of male pollen donor and female seed parent on allocation of resources to developing seeds and fruit in *Chamaecrista fasciculata* (Leguminosae). *American Journal of Botany* 78: 13-23.
- Finnish Forest Research Institute (2004) *Finnish Statistical Yearbook of Forestry*. Vammalan Kirjapaino Oy.

- Flower-Ellis JGK (1971) Age structure and dynamics in stands of bilberry (*Vaccinium myrtillus* L.). PhD thesis, Royal College of Forestry, Stockholm, Department of Forest Ecology and Forest Soils, Research Notes 9: 1-108.
- Fröberg H (1996) Pollination and seed production in five species of *Vaccinium* and *Andromeda* (Ericaceae). Canadian Journal of Botany 74: 1363-1368.
- Galloway LF, Cirigliano T & Gremski K (2002) The contribution of display size and dichogamy to potential geitonogamy in *Campanula americana*. International Journal of Plant Sciences 163: 133-139.
- Gange AC & Smith AK (2005) Arbuscular mycorrhizal fungi influence visitation rates of pollinating insects. Ecological Entomology 30: 600-606.
- Ganger MT (1997) The influence of pollen addition and ramet isolation on current sexual reproduction in a clonal herb. Oecologia 110: 231-236.
- García D, Zamora R, Gómez JM, Jordano P & Hódar JA (2000) Geographical variation in seed production, predation and abortion in *Juniperus communis* throughout its range in Europe. Journal of Ecology 88: 436-446.
- García MB & Ehrlén J (2002) Reproductive effort and herbivory timing in a perennial herb: fitness components at the individual and population levels. American Journal of Botany 89: 1295-1302.
- Geber MA, Dawson TE & Delph LF (1999) Gender and sexual dimorphism in flowering plants. Springer-Verlag, Berlin, Heidelberg, Germany.
- Goodwillie C (1999) Multiple origins of self-compatibility in *Linanthus* section *Leptosiphon* (Polemoniaceae): phylogenetic evidence from internal-transcribed-spacer sequence data. Evolution 53: 1387-1395.
- Goodwillie C, Partis KL & West JW (2004) Transient self-incompatibility confers delayed selfing in *Leptosiphon jepsonii* (Polemoniaceae). International Journal of Plant Sciences 165: 387-394.
- Goodwillie C, Kalisz S & Eckert CG (2005) The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. Annual Review of Ecology, Evolution, and Systematics 36: 47-79.
- Graham JH, Druillard DL & Hodge NC (1996) Carbon economy of sour orange in response to different *Glomus* spp. Tree Physiology 16: 1023-1029.
- Guillaume P & Jacquemart A-L (1999) Early-inbreeding depression in *Vaccinium myrtillus* and *V. vitis-idaea*. Protoplasma 208: 107-114.
- Handel SN (1983) Pollination ecology, plant population structure, and gene flow. In: Real L (ed) Pollination biology. Academic Press, New York, New York, USA, 163-211.
- Handel SN (1985) The intrusion of clonal growth patterns on plant breeding systems. American Naturalist 125: 367-384.
- Harder LD & Barrett SCH (1995) Mating cost of large floral displays in hermaphrodite plants. Nature 373: 512-515.
- Harley JL & Harley EL (1987) A check-list of mycorrhiza in the British flora. New Phytologist 105: 1-102.

- Hautala H, Tolvanen A & Nuortila C (2001) Regeneration strategies of dominant boreal forest dwarf shrubs in response to selective removal of understory layers. *Journal of Vegetation Science* 12: 503-510.
- Helenurm K & Barrett SCH (1987) The reproductive biology of boreal forest herbs. II. Phenology of flowering and fruiting. *Canadian Journal of Botany* 65: 2047-2056.
- Helenurm K & Schaal BA (1996) Genetic load, nutrient limitation, and seed production in *Lupinus texensis* (Fabaceae). *American Journal of Botany* 83: 1585-1595.
- Hellström K, Kytöviita M-M, Tuomi J & Rautio P (2006) Plasticity of clonal integration in the perennial herb *Linaria vulgaris* after damage. *Functional Ecology* 20: 413-420.
- Hemborg ÅM (1998) Costs of reproduction in subarctic *Ranunculus acris*: a five-year field experiment. *Oikos* 83: 273-282.
- Hendrix SD (1988) Herbivory and its impact on plant reproduction. In: Lovett Doust JL & Lovett Doust LL (eds) *Plant reproductive ecology: patterns and strategies*. Oxford University Press, New York, New York, USA, 246-263.
- Heppell KB & Shumway DL & Koide RT (1998) The effect of mycorrhizal infection of *Abutilon theophrasti* on competitiveness of offspring. *Functional Ecology* 12: 171-175.
- Herrera J (1993) Blooming times of individual inflorescences and plants as determinants of flower and fruit predation in *Lavandula stoechas* (Lamiaceae). *Acta Oecologica* 14: 867-874.
- Hill-Cottingham DG & Williams RR (1967) Effect of time of application of fertilizer nitrogen on the growth, flower development, and fruit set of maiden apple trees, var. Lord Lambourne, and on the distribution of total nitrogen within the trees. *Journal of Horticultural Science* 42: 319-338.
- Hokanson K & Hancock J (2000) Early-acting inbreeding depression in three species of *Vaccinium* (Ericaceae). *Sexual Plant Reproduction* 13: 145-150.
- Holsinger K (1991) Inbreeding depression and the evolution of plant mating systems. *Trends in Ecology and Evolution* 6: 307-308.
- Houle G (2001) Reproductive costs are associated with both the male and female functions in *Alnus viridis* ssp. *crispa*. *Ecoscience* 8: 220-229.
- Ingestad T (1979) Mineral nutrient requirements of *Pinus silvestris* and *Picea abies* seedlings. *Physiologia Plantarum* 45: 373-380.
- Jacquemart A-L (1993) Floral visitors of *Vaccinium* species in the High Ardennes, Belgium. *Flora* 188: 263-273.
- Jacquemart A-L & Thompson JD (1996) Floral and pollination biology of three sympatric *Vaccinium* (Ericaceae) species in the Upper Ardennes, Belgium. *Canadian Journal of Botany* 74: 210-221.
- Jacquemart A-L, Mahy G, Raspé O & De Sloover JR (1994) An isozyme study in bilberry (*Vaccinium myrtillus*), 2. Mating system and genetic structure. *Belgian Journal of Botany* 127: 105-114.
- de Jong TJ, Waser NM & Klinkhamer PGL (1993) Geitonogamy: the neglected side of selfing. *Trends in Ecology and Evolution* 8: 321-325.

- Kärkkäinen K, Koski V & Savolainen O (1996) Geographical variation in the inbreeding depression of Scots pine. *Evolution* 50: 111-119.
- Klekowski EJ (1988) Genetic load and its causes in long-lived plants. *Trees* 2: 195-203.
- Klekowski EJ & Godfrey PJ (1989) Ageing and mutation in plants. *Nature* 340: 389-391.
- Knight TM (2003) Floral density, pollen limitation, and reproductive success in *Trillium grandiflorum*. *Oecologia* 144: 557-563.
- Knuth P (1899) *Handbuch der Blütenbiologie*. Bd. II.2. Verlag Wilhelm Engelmann, Leipzig, Germany.
- Koide RT (1991) Nutrient supply, nutrient demand and plant response to mycorrhizal infection. *New Phytologist* 117: 365-386.
- Koide RT (2000) Mycorrhizal symbiosis and plant reproduction. In: Kapulnik Y & Douds DD Jr (eds) *Arbuscular mycorrhizas: physiology and function*. Kluwer Academic Publishers, The Netherlands, 19-46.
- Koide RT, Li M, Lewis J & Cherie I (1988) Role of mycorrhizal infection in the growth and reproduction of wild vs. cultivated plants. I. wild vs. cultivated oats. *Oecologia* 77: 537-543.
- Krebs SL & Hancock JF (1990) Early acting inbreeding depression and reproductive success in the highbush blueberry, *Vaccinium corymbosum* L. *Theoretical and Applied Genetics* 79: 825-832.
- Krebs SL & Hancock JF (1991) Embryonic genetic load in the highbush blueberry, *Vaccinium corymbosum* (Ericaceae). *American Journal of Botany* 78: 1427-1437.
- Kytöviita MM, Vestberg M & Tuomi J (2003) A test of mutual aid in common mycorrhizal networks: established vegetation negates benefit in seedlings. *Ecology* 84: 898-906.
- Lande R & Schemske DW (1985) The evolution of self-fertilization and inbreeding depression in plants. I. Genetic Models. *Evolution* 39: 24-40.
- Lau T-C & Stephenson AG (1993) Effects of soil nitrogen on pollen production, pollen grain size, and pollen performance in *Cucurbita pepo* (Cucurbitaceae) *American Journal of Botany* 80: 763-768.
- Lau T-C, Lu X, Koide RT & Stephenson AG (1995) Effects of soil fertility and mycorrhizal infection on pollen production and pollen grain size of *Cucurbita pepo* (Cucurbitaceae). *Plant, Cell and Environment* 18: 169-177.
- Leclerc-Potvin C & Ritland K (1994) Modes of self-fertilization in *Mimulus guttatus* (Scrophulariaceae): a field experiment. *American Journal of Botany* 81: 199-205.
- Lehtilä K & Syrjänen K (1995) Positive effects of pollination on subsequent size, reproduction, and survival of *Primula veris*. *Ecology* 76: 1084-1098.
- Lehtilä K & Strauss SY (1999) Effects of foliar herbivory on male and female reproductive traits of wild radish, *Raphanus raphanistrum*. *Ecology* 80: 116-124.
- Levin DA (1981) Dispersal versus gene flow in plants. *Annals of the Missouri Botanical Garden* 68: 233-253.
- Levin DA (1984) Inbreeding depression and proximity-dependent crossing success in *Phlox drummondii*. *Evolution* 38: 116-127.

- Levin DA (1989) Proximity-dependent cross-compatibility in *Phlox*. *Evolution* 43: 1114-1116.
- Levri MA (1998) The effect of timing of pollination on the mating system and fitness of *Kalmia latifolia* (Ericaceae). *American Journal of Botany* 85: 1626-1630.
- Levri MA & Real LA (1998) The role of resources and pathogens in mediating the mating system of *Kalmia latifolia*. *Ecology* 79: 1602-1609.
- Lewis JD & Koide RT (1990) Phosphorus supply, mycorrhizal infection and plant offspring vigour. *Functional Ecology* 4: 695-702.
- Lloyd DG & Schoen DJ (1992) Self- and cross-fertilization in plants. I. Functional dimensions. *International Journal of Plant Sciences* 153: 358-369.
- Lord J (1998) Effect of flowering on vegetative growth and further reproduction in *Festuca novae-zelandiae*. *New Zealand Journal of Ecology* 22: 25-31.
- Lu X & Koide RT (1994) The effects of mycorrhizal infection on components of plant growth and reproduction. *New Phytologist* 128: 211-218.
- Lynch M & Walsh B (1998) Genetics and analysis of quantitative traits. Sinauer Associates, Sunderland, Mass, 251-291.
- Mahy G & Jacquemart A-L (1998) Mating system of *Calluna vulgaris*: self-sterility and outcrossing estimations. *Canadian Journal of Botany* 76: 37-42.
- Mahy G & Jacquemart A-L (1999) Early inbreeding depression and pollen competition in *Calluna vulgaris* (L.) Hull. *Annals of Botany* 83: 697-704.
- Martin ME & Lee TD (1993) Self pollination and resource availability affect ovule abortion in *Cassia fasciculata* (Caesalpiniaceae). *Oecologia* 94: 503-509.
- Molau U, Carlsson M, Dahlberg A & Hill Ö (1989) Mating system and pollen-mediated gene flow in *Bartsia alpina*. *Oikos* 55: 409-419.
- Mutikainen P & Ojala A (1993) Simulated herbivory and air pollution: growth and reproduction of an evergreen dwarf shrub, *Empetrum nigrum*. *Acta Oecologica* 14: 771-780.
- Mutikainen P & Delph LF (1996) Effects of herbivory on male reproductive success in plants. *Oikos* 75: 353-358.
- Newport MEA (1989) A test for proximity-dependent outcrossing in the alpine skypilot, *Polemonium viscosum*. *Evolution* 43: 1110-1113.
- Ng S-C & Corlett RT (2000) Comparative reproductive biology of the six species of *Rhododendron* (Ericaceae) in Hong Kong, South China. *Canadian Journal of Botany* 78: 221-229.
- Nousiainen H (1980) Eräiden *Vaccinium*-lajien ja suokukan pöytysbiologiasta. MSc thesis, Department of Botany, University of Helsinki. In Finnish.
- Nyman Y (1992) Pollination mechanisma in six *Campanula* species (Campanulaceae). *Plant Systematics and Evolution* 181: 97-108.
- Nyman Y (1993) The pollen-collecting hairs of *Campanula* (Campanulaceae). II. Function and adaptive significance in relation to pollination. *American Journal of Botany* 80: 1437-1443.
- Obeso JR (1993a) Cost of reproduction in the perennial herb *Asphodelus albus* (Liliaceae). *Ecography* 16: 365-371.

- Obeso JR (1993b) Does defoliation affect reproductive output in herbaceous perennials and woody plants in different ways? *Functional Ecology* 7: 150-155.
- Obeso JR (2002) The costs of reproduction in plants. *New Phytologist* 155: 321-348.
- Parrish JAD & Bazzaz FA (1985) Nutrient content of *Abutilon theophrasti* seeds and the competitive ability of the resulting plants. *Oecologia* 65: 247-251.
- Peng S, Eissenstat DM, Graham JH, Williams K & Hodge NC (1993) Growth depression in mycorrhizal citrus at high-phosphorus supply. *Plant Physiology* 101: 1063-1071.
- Philip LJ, Poluszny U & Klironomos JN (2001) The influence of mycorrhizal colonization on the vegetative growth and sexual reproductive potential of *Lythrum salicaria* L. *Canadian Journal of Botany* 79: 381-388.
- Pitelka LF & Ashmun JW (1985) Physiology and integration of ramets in clonal plants. In: Jackson JBC, Buss LW & Cook RE (eds) *Population biology and evolution of clonal organisms*. Yale University Press, New Haven and London, USA, 399-435.
- Pojar J (1974) Reproductive dynamics of four plant communities of south-western British Columbia. *Canadian Journal of Botany* 52: 1819-1834.
- Popova TN (1972) *Vaccinium* L. In Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM & Webb DA (eds) *Flora Europaea*. Vol. 3. Cambridge University Press, Cambridge, UK, 12-13.
- Poulton JL, Koide RT & Stephenson AG (2001) Effects of mycorrhizal infection and soil phosphorus availability on in vitro and in vivo pollen performance in *Lycopersicon esculentum* (Solanaceae). *American Journal of Botany* 88: 1786-1793.
- Poulton JL, Bryla D, Koide RT, Stephenson AG (2002) Mycorrhizal infection and high soil phosphorus improve vegetative growth and the female and male functions in tomato. *New Phytologist* 154: 255-264.
- Price MV & Waser NM (1979) Pollen dispersal and optimal outcrossing in *Delphinium nelsoni*. *Nature* 277: 294-297.
- Primack RB & Hall P (1990) Costs of reproduction in the pink lady's slipper orchid: a four-year experimental study. *American Naturalist* 136: 638-656.
- Primack R & Stacy E (1998) Cost of reproduction in the pink lady's slipper orchid (*Cypripedium acaule*, Orchidaceae): an eleven-year experimental study of three populations. *American Journal of Botany* 85: 1672-1679.
- Primack RB, Miao SL & Becker KR (1994) Costs of reproduction in the pink lady's slipper orchid: defoliation, increased fruit production, and fire. *American Journal of Botany* 81: 1083-1090.
- Raspé O, Guillaume P & Jacquemart A-L (2004) Inbreeding depression and biased paternity after mixed-pollination in *Vaccinium myrtillus* L. (Ericaceae). *International Journal of Plant Sciences* 165: 765-771.
- Rathcke B (1988) Interactions for pollination among coflowering shrubs. *Ecology* 69: 446-457.
- Rathcke B & Real L (1993) Autogamy and inbreeding depression in Mountain Laurel, *Kalmia latifolia* (Ericaceae). *American Journal of Botany* 80: 143-146.

- Rautio P, Huhta A-P, Piippo S, Tuomi J, Juenger T, Saari M & Aspi J (2005) Overcompensation and adaptive plasticity of apical dominance in *Erysimum strictum* (Brassicaceae) in response to simulated browsing and resource availability. *Oikos* 111: 179-191.
- Reader RJ (1977) Bog ericad flowers: self-compatibility and relative attractiveness to bees. *Canadian Journal of Botany* 55: 2279-2287.
- Reekie EG & Bazzaz FA (1987) Reproductive effort in plants. III. Effect of reproduction on vegetative activity. *American Naturalist* 129: 907-919.
- Reekie EG & Bazzaz FA (1992) Cost of reproduction as reduced growth in genotypes of two congeneric species with contrasting life histories. *Oecologia* 90: 21-26.
- Reinartz JA & Les DH (1994) Bottleneck-induced dissolution of self-incompatibility and breeding system consequences in *Aster furcatus* (Asteraceae). *American Journal of Botany* 81: 446-455.
- Richards AJ (1997) Plant breeding systems. 2nd ed, Chapman & Hall, UK.
- Ritchie JC (1955) Biological flora of the British Isles: *Vaccinium vitis-idaea* L. *Journal of Ecology* 43: 701-708.
- Ritchie JC (1956) Biological flora of the British Isles: *Vaccinium myrtillus* L. *Journal of Ecology* 44: 291-299.
- Roach DA & Wulff RD (1987) Maternal effects in plants. *Annual Review of Ecology and Systematics* 18: 209-235.
- Roff DA (1992) Evolution of life-histories, theory and analysis. Chapman & Hall, New York, Inc.
- Sachs T & Novoplansky A (1997) What does a clonal organization suggest concerning clonal plants? In: de Kroon H & van Groenendael J (eds) The ecology and evolution of clonal plants. Backhuys Publishers, Leiden, The Netherlands, 55-77.
- Salemaa M & Sievänen R (2002) The effect of apical dominance on the branching architecture of *Arctostaphylos uva-ursi* in four contrasting environments. *Flora* 197: 429-442.
- Santandreu M & Lloret F (1999) Effect of flowering phenology and habitat on pollen limitation in *Erica multiflora*. *Canadian Journal of Botany* 77: 734-743.
- Schemske DW & Lande R (1985) The evolution of self-fertilization and inbreeding depression in plants. II. Empirical observations. *Evolution* 39: 41-52.
- Schemske DW & Pautler LP (1984) The effects of pollen composition of fitness components in a neotropical herb. *Oecologia* 62: 31-36.
- Schlichting CD & Devlin B (1992) Pollen and ovule sources affect seed production of *Lobelia cardinalis* (Lobeliaceae). *American Journal of Botany* 79: 891-898.
- Schmitt J (1980) Pollinator foraging behavior and gene dispersal in *Senecio* (Compositae). *Evolution* 34: 934-943.
- Schoen DJ, Johnston MO, L'Heureux A-M & Marsolais JV (1997) Evolutionary history of the mating system in *Amsinckia* (Boraginaceae). *Evolution* 51: 1090-1099.
- Seavey SR & Carter S-K (1994) Self-sterility in *Epilobium obcordatum* (Onagraceae). *American Journal of Botany* 81: 331-338.

- Selås V (2000) Seed production of a masting dwarf shrub, *Vaccinium myrtillus*, in relation to previous reproduction and weather. *Canadian Journal of Botany* 78: 423-429.
- Silvertown J & Charlesworth D (2001) Introduction to plant population biology. 4th ed. Blackwell Science, Oxford, UK.
- Shefferson RP, Kull T & Tali K (2006) Demographic response to shading and defoliation in two woodland orchids. *Folia Geobotanica* 41: 95-106
- Sorensen F (1969) Embryonic genetic load in coastal Douglas-Fir, *Pseudotsuga menziesii* var. *menziesii*. *American Naturalist* 103: 389-398.
- Stanley MR, Koide RT & Shumway DL (1993) Mycorrhizal symbiosis increases growth, reproduction and recruitment of *Abutilon theophrasti* Medic. in the field. *Oecologia* 94: 30-35.
- Stearns SC (1989) Trade-offs in life-history evolution. *Functional Ecology* 3: 259-268.
- Stearns SC & Hoekstra RF (2000) Evolution: an introduction. Oxford University Press Inc., New York, USA.
- Stephenson AG, Winsor JA, Richardson TE, Singh A & Kao T-H (1992) Effects of style age on the performance of self and cross pollen in *Campanula rapunculoides*. In: Ottaviano E, Mulcahy DL, Sari Gorla M & Bergamini Mulcahy G (eds) Angiosperm pollen and ovules. Springer-Verlag, New York, 117-121.
- Sultan SE (1996) Phenotypic plasticity for offspring traits in *Polygonum persicaria*. *Ecology* 77: 1791-1807.
- Sutherland S & Delph L (1984) On the importance of male fitness in plants: patterns of fruit set. *Ecology* 65: 1093-1104.
- Syrjänen K & Lehtilä K (1993) The cost of reproduction in *Primula veris*: differences between two adjacent populations. *Oikos* 67: 465-472.
- Thórhallsdóttir TE (1998) Flowering phenology in the central highland of Iceland and implications for climatic warming in the Arctic. *Oecologia* 114: 43-49.
- Tolvanen A (1994) Differences in recovery between a deciduous and an evergreen ericaceous clonal dwarf shrub after simulated aboveground herbivory and belowground damage. *Canadian Journal of Botany* 71: 853-859.165.
- Tolvanen A & Laine K (1997) Effects of reproduction and artificial herbivory on vegetative growth and resource levels in deciduous and evergreen dwarf shrubs. *Canadian Journal of Botany* 75: 656-666.
- Totland O (1994) Intra-seasonal variation in pollination intensity and seed set in an alpine population of *Ranunculus acris* in southwestern Norway. *Ecography* 17: 159-165.
- Totland O, Nielsen A, Bjerken A-L, & Ohlson M (2006) Effects of an exotic plant and habitat disturbance on pollinator visitation and reproduction in a boreal forest herb. *American Journal of Botany* 93: 868-873.
- Trame A-M, Coddington AJ & Paige KN (1995) Field and genetic studies testing optimal outcrossing in *Agave schottii*, a long-lived clonal plant. *Oecologia* 104: 93-100.
- Travers SE, Mena-Ali J & Stephenson, AG (2004) Plasticity in the self-incompatibility system of *Solanum carolinense*. *Plant Species Biology* 19: 127-135.
- Turcotte J & Houle G (2001) Reproductive costs in *Salix planifolia* ssp. *planifolia* in subarctic Québec, Canada. *Ecoscience* 8: 506-512.

- Tuomi J, Hakala T & Haukioja E (1983) Alternative concepts of reproductive effort, cost of reproduction, and selection in life-history evolution. *American Zoologist* 23: 25-34.
- Vander Kloet SP & Lyrene PM (1987) Self-incompatibility in diploid, tetraploid, and hexaploid *Vaccinium corymbosum*. *Canadian Journal of Botany* 65: 660-665.
- Vander Kloet SP & Hill NM (1994) The paradox of berry production in temperate species of *Vaccinium*. *Canadian Journal of Botany* 72: 52-58.
- Viramo J (1978) Experiments on the pollination biology of the bilberry and red whortle berry in Kuusamo. *Acta Univ Oul A* 68: Biol 4: 195-207. In Finnish with English summary.
- Vogler DW, Das C & Stephenson AG (1998) Phenotypic plasticity in the expression of self-incompatibility in *Campanula rapunculoides* L. *Heredity* 81: 546-555.
- Waddington KD (1983) Foraging behavior of pollinators. In: Real L (ed) *Pollination biology*. Academic Press, Orlando, Florida, USA, 213-239.
- Wyatt R (1983) Pollinator-plant interactions and the evolution of breeding systems. In: Real L (ed) *Pollination biology*. Academic Press, Orlando, Florida, USA, 51-95.
- Waser NM & Price MV (1989) Optimal outcrossing in *Ipomopsis aggregata*: seed set and offspring fitness. *Evolution* 43: 1097-1109.
- Waser NM & Price MV (1991) Outcrossing distance effects in *Delphinium nelsoni*: pollen loads, pollen tubes, and seed set. *Ecology* 72: 171-179.
- Welch D, Scott D & Doyle S (2000) Studies on the paradox of seedling rarity in *Vaccinium myrtillus* L. in NE Scotland. *Botanical Journal of Scotland* 52: 17-30.
- Wijesinghe DK, John EA, Beurskens S & Hutchings MJ (2001) Root system size and precision in nutrient foraging: responses to spatial pattern of nutrient supply in six herbaceous species. *Journal of Ecology* 89: 972-983.
- Wolfe BE, Husband BC & Klironomos JN (2005) Effects of a belowground mutualism on an aboveground mutualism. *Ecology Letters* 8: 218-223.
- Wood GW (1962) The period of receptivity in flowers of the lowbush blueberry. *Canadian Journal of Botany* 40: 685-686.
- Wyka T & Galen C (2000) Current and future costs of reproduction in *Oxytropis sericea*, a perennial plant from the Colorado Rocky Mountains, U.S.A. *Arctic, Antarctic and Alpine Research* 32: 438-448.
- Young HJ & Stanton ML (1990) Influence of environmental quality on pollen competitive ability in wild radish. *Science* 248: 1631-1633.
- Zvereva EL & Kozlov MV (2005) Growth and reproduction of dwarf shrubs, *Vaccinium myrtillus* and *V. vitis-idaea*, in a severely polluted area. *Basic and Applied Ecology* 6: 261-274.

Appendix 1

Table A1. Autofertility (AFI) and self-fertility indices (SFI) for reviewed Ericaceae plant species (compare Lloyd & Schoen 1992). The values are based on fruit set, seed set data or number of seeds per fruit. The latter cases are marked with an asterisk. Data source: 1: the values were calculated from the data given in the original reference; 2: the values are cited from the original reference. Unless otherwise mentioned, the studies were conducted in the field.

Species name	Data source	AFI/		SFI/		Place of investigation	Reference
		Fruit set	Seed set	Fruit set	Seed set		
<i>Andromeda glaucophylla</i>	1	0		1.0		Canada	Reader 1977
<i>A. polifolia</i>	1	0.73	0.39	0.49	0.19	Central Sweden	Fröberg 1996
<i>Calluna vulgaris</i>	1	0.11	0.02 *	0.48	0.13 *	Belgium, greenhouse	Mahy & Jacquemart 1998
<i>Chamaedaphne calyculata</i>	1	0, 0		0.86, 1		Canada, two sites	Reader 1977
<i>Erica andevalensis</i>	1	0		1.0	0.89 *	Spain, greenhouse	Aparicio & Garcia-Martin 1996
<i>E. multiflora</i>	1			0.40	0.27 *	Spain	Santandreu & Lloret 1999
<i>Gaultheria procumbens</i>	1	0		0.88		Canada	Reader 1977
<i>Kalmia angustifolia</i>	1	0.84		0.81		Rhode Island, USA	Rathcke 1988
<i>K. latifolia</i>	1	0.12			0.35 *	Virginia, USA	Levri 1998
<i>K. latifolia</i>	1	0.02,		0.23,		Virginia, USA	Levri & Real 1998
<i>K. latifolia</i>	1	0.21		0.23		Rhode Island and Virginia, USA	Rathcke & Real 1993
<i>K. polifolia</i>	1	0		0.9		Canada	Reader 1977
<i>Ledum groenlandicum</i>	1	0		1.0		Canada	Reader 1977
<i>Rhododendron</i>	1, 2	0.02	0	0.2	0	Hong Kong	Ng & Corlett 2000
<i>championiae</i>							
<i>R. farrerae</i>	1, 2	0.07	0	0.83	0.18	Hong Kong	Ng & Corlett 2000
<i>R. ferrugineum</i>	2		0.92 *		0.96 *	French Alps	Escaravage <i>et al.</i> 1997
<i>R. hongkongense</i>	1, 2	0.04	0	0.61	0.11	Hong Kong	Ng & Corlett 2000

Table A1 (Continued).

Species name	Data source	AFI/		SFI/		Place of investigation	Reference
		Fruit set	Seed set	Fruit set	Seed set		
<i>R. moulinhense</i>	1, 2	0	0	0	0	Hong Kong	Ng & Corlett 2000
<i>R. simiarum</i>	1, 2	0.14	0	0.91	0.08	Hong Kong	Ng & Corlett 2000
<i>R. simsii</i>	1, 2	0.06	0	0.52	0.05	Hong Kong	Ng & Corlett 2000
<i>Vaccinium macrocarpon</i>	1	0		0.89		Canada	Reader 1977
<i>V. myrtilloides</i>	1	0		0.57		Canada	Reader 1977
<i>V. myrtilloides</i>	1	0.12	0.67	0.07	0.12 *	USA, greenhouse	Hokanson & Hancock 2000
<i>V. myrtilus</i>	1	0.66,	0.32 *	0.13	0.46	Central Sweden	Fröberg 1996
<i>V. myrtilus</i>	2	0.35	0.25 *	1.12,	0.52 *	Belgium, two successive years	Jacquemart & Thompson 1996
				1.11	0.56 *		
<i>V. myrtilus</i>	1			0.79	0.19 *	Belgium, greenhouse	Guillaume & Jacquemart 1999
<i>V. myrtilus</i>	1	0.01,		0.68,	0.22 *	Finland, two sites	present work, paper IV
		0		0.57	0.26 *		
<i>V. oxycoccus</i>	1	0		0.89		Canada	Reader 1977
<i>V. oxycoccus</i>	1	0.32		0.32		Central Sweden	Fröberg 1996
<i>V. uliginosum</i>	1	0		0		Central Sweden	Fröberg 1996
<i>V. uliginosum</i>	2	0.18,	0.43 *	1.06,	0.84 *	Belgium, two successive years	Jacquemart & Thompson 1996
		0.23	0.30 *	0.47	0.41 *		
<i>V. vitis-idaea</i>	1	0.17	0.39	0.2	0.37	Central Sweden	Fröberg 1996
<i>V. vitis-idaea</i>	2	0.23	0.22 *	0.84	0.36 *	Belgium	Jacquemart & Thompson 1996
<i>V. vitis-idaea</i>	1			0.68	0.25 *	Belgium, greenhouse	Guillaume & Jacquemart 1999
<i>V. vitis-idaea</i>	1	0		0.75	0.28 *	Finland	present work, paper IV

Original papers

- I Nuortila C. Little variation in fruit/flower ratio between years in two ericaceous dwarf shrubs, *Vaccinium myrtillus* and *Vaccinium vitis-idaea*. Manuscript.
- II Nuortila C, Tuomi J & Tolvanen A. Cost of sexual reproduction on future reproduction and vegetative growth in evergreen *Vaccinium vitis-idaea* and deciduous *Vaccinium myrtillus*. Manuscript.
- III Nuortila C, Tuomi J, Aspi J & Laine K (2006) Early-acting inbreeding depression in a clonal dwarf shrub, *Vaccinium myrtillus*, in a northern boreal forest. *Annales Botanici Fennici* 43: 36-48.
- IV Nuortila C, Tuomi J & Laine K (2002) Inter-parent distance affects reproductive success in two clonal dwarf shrubs, *Vaccinium myrtillus* and *Vaccinium vitis-idaea* (Ericaceae). *Canadian Journal of Botany* 80: 875-884.
- V Nuortila C, Kytöviita M-M & Tuomi J (2004) Mycorrhizal symbiosis has contrasting effects on fitness components in *Campanula rotundifolia*. *New Phytologist* 164: 543-553.

The articles are reprinted with kind permission of the Finnish Zoological and Botanical Publishing Board (III), the NRC Research Press (IV) and the New Phytologist (V).

Original articles are not included in the electronic version of the dissertation.

474. Miinalainen, Ilkka (2006) Enoyl thioester reductases—enzymes of fatty acid synthesis and degradation in mitochondria
475. Klaavuniemi, Tuula (2006) PDZ-LIM domain proteins and α -actinin at the muscle Z-disk
476. Reini, Kaarina (2006) Characterisation of the human DNA damage response and replication protein Topoisomerase II β Binding Protein I (TopBPI)
477. Pudas, Regina (2006) Structural and interaction studies on the carboxy-terminus of filamin, an actin-binding protein
478. Hillukkala, Tomi (2006) Roles of DNA polymerase epsilon and TopBPI in DNA replication and damage response
479. Mäki-Petäys, Hannaleena (2007) Conservation and management of populations in a fragmented forest landscape. Behavioural ecology meets population genetics
480. Kärkkäinen, Johanna (2007) Preparation and characterization of some ionic liquids and their use in the dimerization reaction of 2-methylpropene
481. Junttila, Juho (2007) Clay minerals in response to Mid-Pliocene glacial history and climate in the polar regions (ODP, Site 1165, Prydz Bay, Antarctica and Site 911, Yermak Plateau, Arctic Ocean)
482. Sipilä, Laura (2007) Expression of lysyl hydroxylases and functions of lysyl hydroxylase 3 in mice
483. Kivimäki, Anri (2007) Wireless telecommunication standardization processes—actors' viewpoint
484. Räisänen, Liisa (2007) Phage-host interactions in *Lactobacillus delbrueckii*: host recognition and transcription of early phage genes
485. Parviainen, Timo (2007) Ruokohelpiviljelyn optimointi suopohjilla. Turvetuotantoalueiden geologisen ympäristön, pohjaturpeen sekä kierrätyslannoitteiden käytön vaikutus ruokohelpin käyttämiin alkuaineisiin ja satoon
486. Halonen, Raija (2007) Challenges in an inter-organisational information system implementation. Participatory view
487. Välimäki, Panu (2007) Reproductive tactics in butterflies – the adaptive significance of monandry versus polyandry in *Pieris napi*
488. Oinas, Janne (2007) The degree theory and the index of a critical point for mappings of the type (S+)

Book orders:
OULU UNIVERSITY PRESS
P.O. Box 8200, FI-90014
University of Oulu, Finland

Distributed by
OULU UNIVERSITY LIBRARY
P.O. Box 7500, FI-90014
University of Oulu, Finland

S E R I E S E D I T O R S

A
SCIENTIAE RERUM NATURALIUM
Professor Mikko Siponen

B
HUMANIORA
Professor Harri Mantila

C
TECHNICA
Professor Juha Kostamovaara

D
MEDICA
Professor Olli Vuolteenaho

E
SCIENTIAE RERUM SOCIALIUM
Senior Assistant Timo Latomaa

E
SCRIPTA ACADEMICA
Communications Officer Elna Stjerna

G
OECONOMICA
Senior Lecturer Seppo Eriksson

EDITOR IN CHIEF
Professor Olli Vuolteenaho

EDITORIAL SECRETARY
Publications Editor Kirsti Nurkkala

ISBN 978-951-42-8499-1 (Paperback)

ISBN 978-951-42-8500-4 (PDF)

ISSN 0355-3191 (Print)

ISSN 1796-220X (Online)

