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Reproductive ecology of Canada mayflower (Maianthemum canadense Desf)

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REPRODUCTIVE ECOLOGY OF CANADA MAYFLOWER (MAIANTHEMUM CANADENSE DESF.)

BY

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DISSERTATION

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ABSTRACT

REPRODUCTIVE ECOLOGY OF CANADA MAYFLOWER (MAIANTHEMUM CANADENSE DESF.)

by

Michael Ganger
University of New Hampshire, May, 1998

Canada mayflower (Maianthemum canadense var. canadense Desf.), a rhizomatous perennial herb, was the subject of field experiments investigating the role of several factors on the sexual reproduction of ramets. Mayflower ramets may be either flowering (with 2–3 leaves and a terminal inflorescence consisting of 4–35 perfect flowers) or vegetative (1 leaf).

Pollen addition increased the number of seeds matured by ramets in three out of four years in which pollen level was experimentally manipulated. The lack of compatible pollen is thought to be a major factor limiting seed maturation by ramets.

The act of severing the rhizomes, such that ramets were isolated from the rest of the genet, also limited the number of seeds matured by ramets, but only when pollen was not limiting. In addition, rhizome severing was responsible for a reduction of, on average, 3.2 flowers brought to anthesis per ramet. Rhizome severing did not appear to delay flowering or to decrease the length of time that flowers were receptive to pollen. Rhizome severing also resulted in increased initiation of rhizomes, while the level of pollination (open pollinated, overpollinated, bagged, and vegetative) was independent of rhizome initiation.

The number of seeds matured per ramet was negatively correlated with the mean weight of seeds, indicating that individual ramets were to some extent resource limited with few-seeded ramets maturing heavier seeds than ramets bearing many seeds. There was no difference detected in the mean number of seeds matured by overpollinated ramets flowering for the first and second time.

The number of seeds matured per ramet was related to the identity of the ramet directly acropetal and basipetal on the same rhizome system ("context"). The most common constext was also the one with the lowest seed maturation (no ramet or a dead ramet both acropetal and basipetal). Flowering ramets were older than vegetative ramets and differed in their contexts. Ages also differed with context and permitted a first attempt at inferring ramet and genet development.

INTRODUCTION

Background and Significance

Reproductive systems may be classified as either sexual or asexual (Fisher 1930). Sexual reproduction involves the creation of a novel genotype and is exclusively a "whole-organism" process. Asexual reproduction involves the duplication of an existing genotype and may occur at the level of genomes, organelles, cells, and individuals (Buss 1985). In seed plants, two types of asexual reproduction predominate at the level of the individual: apomixis (the production of asexual seed) and ramet production (Abrahamson 1980). Due to the tendency for newly produced ramets to remain connected with the parent plant for some time, ramet production has been termed clonal growth or clonal reproduction (Jackson, Buss, and Cook 1985).

Clonal reproduction is represented by species in many animal phyla (Hughes and Cancino 1985) and in 10 of the 11 vascular plant classes (Mogie and Hutchings 1990). Clonal plants are represented throughout geological time (Tiffney and Niklas 1985) and are represented in many present-day communities with some communities being dominated ecologically and/or numerically by clonal plant species (Pacala 1989). For example, 70% of plant species in the temperate, deforested zone of the earth's surface are clonal (van Groenendael and de Kroon 1990). Clonal plants also occur in the forested zones. Many trees species are clonal and the vast majority of temperate, understory herbs are clonal as well (Anderson and Loucks 1973, Sobey and Barkhouse 1977, Bierzychudek

1982). Given these statistics, knowledge of how clonal plants grow and reproduce may be crucial to our understanding of the ecology and evolution of plant communities.

Sexual and Clonal Reproduction

The resources available to a plant are likely to vary through time and at any time at least one resource is likely to be limiting (Bazzaz et al. 1987). In any year, a plant must allocate potentially limiting resources to structures associated with growth, reproduction, and defense, while over the lifetime of the plant, resources must be balanced between reproduction and survivorship (Bazzaz et al. 1987).

Clonal plants are rarely exclusively clonal but have the ability to reproduce sexually as well (Silander 1985). Within an individual, resources allocated to one form of reproduction may come at the expense of allocation to the other form of reproduction (Williams 1975) and the pattern of reproductive allocation is likely to differ among genotypes (Watson 1984). If at least a portion of the allocation patterns of plants is heritable and these patterns represent different fitnesses, then over time one form of reproduction would be expected to be lost since genotypes engaging in this form of reproduction would have lower fitnesses.

Clonal reproduction has been touted to have a numerical advantage over sexual reproduction. Individuals engaging in clonal reproduction do not incur the "cost" of producing males and can be shown, at least on paper, to hold a two-factor advantage in offspring production (Maynard Smith 1978). Individuals engaging in clonal reproduction pass all of their genes onto the next generation including gene complexes that may be optimal for the local environment. Sexual reproduction with its recombination and meiosis can result in the loss of such gene complexes (Kondroshov 1988). Indeed, the question generally posed for clonal individuals is "Why have sex?".

Several hypotheses have been offered as to why sexual reproduction is advantageous. Levin (1975) points out that certain genotypes may be more susceptible to particular pests and pathogens. Given such a situation, the ability to recombine genes (sexual reproduction) may represent an advantage. The number of deleterious mutations may accumulate in a population of exclusively clonal individuals and the number of such mutations could only increase over time (termed Muller's ratchet; Maynard Smith 1978). Sexual reproduction, through recombination, can result in the removal of linkage disequilibrium and "restore" beneficial gene complexes (Kondroshov 1988).

Given the relative advantages and disadvantages of each form of reproduction, should not there be ecological situations under which one form of reproduction should be favored to the exclusion of the other? The answer may be that environments are variable enough with respect to both space and time and therefore neither form of reproduction would have a clear advantage for long (Warner 1977). Both forms of reproduction would be predicted to remain, although not in equilibrium. This may be true of a species viewed over ecological time, but what of the land plants viewed over evolutionary time?

The earliest land plants were clonal (Mogie and Hutchings 1990). Over evolutionary time, one form of reproduction has emerged to exclude the other in many cases; that is, there are many plants capable of reproducing sexually but not clonally. In fact the question might better be posed as "Why reproduce clonally?"

Advantages of Clonal Reproduction

Survivorship in plants (and in many animals as well) is generally positively correlated with size (Harper 1977). Offspring, typically small in size, experience higher rates of mortality than adults. The function relating survivorship and size can be such that small increases in size for small individuals may result in much higher survivorship. Sexually produced offspring, beginning independent life as a seedling, must pass through the higher mortality phase in order to eventually reproduce themselves. Clonally reproduced offspring may be supplemented through this high mortality phase by the parent plant such that the probability of surviving to reproduce may be significantly higher (Cook 1979).

In addition to supplementing offspring, the genet, through the activities of specific ramets, may also alter the survivorship and even reproductive activities of other ramets. Resources have been shown to move from older to younger ramets through acropetal translocation (Pitelka and Ashmun 1985). Basipetal translocation has been shown to occur as well during periods of "stress," typically shading or defoliation, experienced by older ramets (Marshall 1990).

A seed may disperse to a location where other individuals of the species do not occur. If that individual cannot reproduce clonally, a new population could not grow. However, if the individual were capable of clonal reproduction, the new population could increase in size. It is also possible that seed from several insect-pollinated individuals could be dispersed into a location outside of the range of its pollinators. Again, clonal reproduction would represent the only method of enlarging the population. This latter case may be rarer, and in fact Levin (1997) presents many examples of self-incompatible outliers that do set seed and of pollinators crossing large distances to outlier populations. Eriksson (1992) suggests that clonal reproduction will be more strongly favored in species with long distance seed dispersal, as clonal reproduction would make genet fitness less dependent on local seed dispersal.

In locations where resources are patchy, clonal reproduction may also have an advantage. Sexually-produced offspring must count on chance to bring them to the location of these resources. Clonally-produced offspring may be dispersed to resource-rich patches if the plant has some method of detecting higher concentrations of resources in the soil (Cook 1983, Salzman 1985, Bazzaz 1991). This may also apply for plants in locations such as the understory of forests where light is patchy (Pearcy 1990).

Clonal plants are ubiquitous and present a challenge to ecologists interested in quanifying reproduction in such plants. Since the growth of clonal populations may occur through the clonal or sexual production of ramets, it may be necessary to address reproductive questions with a clonal plant in which clonal and sexual reproduction are easy to quantify. Canada mayflower (*Maianthemum canadense* Desf.) is such a plant.

Canada Mayflower

Canada mayflower (*Maianthemum canadense* var. *canadense* Desf.; Gleason and Cronquist 1991) is common to the woodlands of eastern North America (Williams 1985). Its range extends north to Labrador and New Foundland, south to Maryland and the mountains of Kentucky and North Carolina, and west to Minnesota (Gleason and Cronquist 1991). The range of var. *canadense* overlaps in eastern Ontario, western New

York, and western Massachusetts with the more central U.S./Canada var. interius Fern. (Gleason and Cronquist 1991).

Carleton and Maycock (1980) found mayflower to inhabit the understory of a variety of canopies in boreal forests. Crowder and Taylor (1984) found a positive association between mayflower and the following canopy species in Ontario: eastern hemlock (Tsuga canadensis), sugar maple (Acer saccharum), and shagbark hickory (Carya ovata). In northern Massachusetts mayflower was found to occur most often in old field white pine (Pinus strobus) sites, however, it also occurred in secondary woodland, broadleaf sites (Whitney and Foster 1988).

In the University of New Hampshire woodlands, Durham, NH, mayflower is common under canopies that have any of the following: eastern hemlock (T. canadensis), red oak (Quercus rubra), red maple (Acer rubrum), sugar maple (A. saccharum), American beech (Fagus grandifolia), black birch (Betula lenta), white ash (Fraxinus americana), and shagbark hickory (C. ovata) (Ganger, personal observation). Also in the U.N.H. woodlands, mayflower co-occurs with a number of understory perennials including wild sasparilla (Aralia nudicaulis), starflower (Trientalis borealis), Solomon's seal (Polygonatum biflorum), partridge berry (Mitchella repens), poison ivy (Toxicodendron radicans), and Clintonia borealis (Ganger, personal observation). Each of these understory plants is clonal as well.

Mayflower reproduces clonally through the production of a monopodial rhizome. At some point in its development, the rhizome turns upward and produces an overwintering bud that will bcome a vegetative ramet in the following year. Mayflower reproduces sexually through the production of seeds. A more detailed explanation of mayflower reproduction will be presented in the relevant chapters. Densities of mayflower in the U.N.H. woodlands can approach 250 ramets per m^2 and mayflower has proven itself to be quite amenable to experimental manipulation.

In this dissertation the following broad question is addressed—what factors influence the success of sexual reproduction in mayflower? Several field experiments were performed to test specific hypotheses concerning the number of seeds matured by mayflower ramets.

The investigations with mayflower began with an attempt to determine if mayflower ramets are essentially independent of one another with respect to sexual reproduction and whether or not a lack of pollen could explain differences in fruit and seed among ramets. A two-year field experiment, described in Chapter I, was used to determine if sexual reproduction was pollen limited and whether there was physiological integration such that ramets connected to the genet were more successful than ramets severed from the genet. Also investigated was whether there was a difference in fruit and seed production of ramets between the two years and whether patterns of ramet reproductive success were similar between years.

Any differences in the number of seeds matured by ramets observed in the Chapter I experimental treatments may be explained by resource limitation at the time of fruit initiation. It is also possible that resource limitation occurred prior to or at the time of flowering and therefore a negative impact on specific aspects of flowering may have been responsible for the observed differences in the number of seeds matured by ramets. In Chapter II, data from a field experiment were analyzed to determine if the act of severing the rhizome of a ramet had a negative impact on the number of flowers brought to anthesis, the timing and duration of anthesis, and therefore the number of seeds matured. Also investigated was whether severing had a positive impact on rhizome initiation.

The question of whether mayflower ramets are essentially independent of other ramets with respect to the number of seeds they mature and whether ramets themselves bear the cost of previous flowering was addressed. In Chapter III a field experiment was performed to test whether ramets flowering for the first time differed in the number of seeds that they matured compared to ramets flowering for the second time. Also addressed was whether the number of seeds matured by ramets was correlated with the mean weight of these seeds and whether this relationship was similar for ramets flowering for the first and second time.

As more and more evidence pointed toward the role of the genet in the number of seeds matured by individual ramets, an attempt was made to quantify the genet. In Chapter IV the concept of "context" was introduced. On a monopodial rhizome system, the context of any ramet may be defined as the identities of the ramets directly acropetal and basipetal on the same rhizome. A study was conducted to determine whether the number of seeds matured ramets could be predicted from context or from ramet-specific variables such as the local density of flowering ramets, the nearest-neighbor flowering ramet, the number of times that the ramet had flowered, and the number of years prior to or since flowering. The context of both vegetative and flowering ramets was explored. Whether vegetative and flowering ramets differed in ages was also investigated

The number of seeds matured by ramets was correlated with their context. Therefore it may have been possible for a ramet's context to ameliorate stresses, such as shading, experienced by the ramet. In Chapter V, a field experiment was undertaken to determine if the number of seeds matured by ramets was related to context, pollen addition, and shading. This experiment was much larger than the previous study and was able to further explore whether vegetative and flowering ramets differed in their contexts and their ages. Whether contexts differed with location was also addressed.

CHAPTER I

THE INFLUENCE OF POLLEN ADDITION AND RAMET ISOLATION ON CURRENT SEXUAL REPRODUCTION IN A CLONAL HERB

Abstract

Canada mayflower (Maianthemum canadense var. canadense Desf.), a rhizomatous, perennial herb, was the subject of a two-year field experiment that examined two factors potentially affecting fruit and seed production: pollen addition and ramet isolation. Ramets were either open pollinated or overpollinated by hand to supplement natural levels. Rhizomes of the ramets were either severed, to prevent resource supplementation from the genet, or left intact. Ramets that were overpollinated matured more fruits and more seeds than ramets that were open pollinated. Thus, mayflower appears to have been pollen limited in both years. Ramets that were open pollinated and whose rhizomes were severed matured as many fruits, seeds, and seeds/fruit as ramets that were open pollinated and whose rhizomes were left intact. Ramets that were overpollinated and whose rhizomes were severed matured fewer fruits, seeds, and seeds/fruit than overpollinated ramets whose rhizomes were left intact. It appears that at natural levels of pollination mayflower ramets are physiologically independent but as the level of pollen increases, mayflower ramets receive support from other parts of the genet.

Introduction

On an ecological level, plants must allocate limited resources to structures associated with growth, defense, and reproduction. On an evolutionary level, allocation patterns must balance fecundity and survivorship over the lifetime of the plant (Bazzaz et al. 1987).

Clonal organisms are rarely exclusively clonal but are capable of reproducing sexually as well (Silander 1985). Because of the dual reproductive habit of clonal plants, resources destined for reproduction may be allocated to either sexual or clonal reproduction, or some combination of the two. Different patterns of allocation among individuals are likely to have ecological significance (Abrahamson 1975) and, if at least some of the patterns of allocation are heritable and represent different fitnesses, are likely to have evolutionary significance (Watson 1984). Since at least one resource may be limiting at a given time, allocation to one form of reproduction is likely to result in lower allocation to an alternative form of reproduction, i.e., a "trade-off" (Williams 1975, Harper 1977). In clonal plants, sexual reproduction and clonal reproduction may operate under phenological constraint with the two modes of reproduction separated temporally (Watson 1990). Decreased allocation to sexual reproduction may result in increased allocation to clonal reproduction and this in turn may affect the allocation to sexual reproduction in subsequent years. Alternatively, the modes of reproduction may co-occur and thus compete directly (Benner and Watson 1989).

The reproductive allocations made by an individual ramet must be placed within the context of the entire genet, which consists of all ramets derived from a single seed. These ramets may or may not be connected to one another. To the extent that they are connected, one ramet may influence the reproductive activities and survivorship of other ramets. Newly produced ramets may be subsidized by older ramets, increasing the survivorship of younger ramets through stressful periods (Cook 1979). Resources have been shown to move from older to younger ramets through acropetal translocation (Pitelka and Ashmun 1985). Basipetal translocation has been shown to occur as well during periods of "stress," typically shading or defoliation, experienced by older ramets (Marshall 1990). Newly produced ramets have also been shown to receive resources, though there is debate as to whether aid is direct from one ramet to another (Hartnett and Bazzaz 1983) or indirect, with ramets depositing and withdrawing resources from a common rhizome (Abrahamson et al. 1991). Ramets are thought to develop with the aid of subsidization, pass into an "adulthood" characterized by minimal if any subsidization, and eventually subsidize other ramets (Pitelka and Ashmun 1985, Marshall 1990, Price and Hutchings 1992).

Canada mayflower (Maianthemum canadense var. canadense Desf.; Gleason and Cronquist 1991) is a rhizomatous, perennial herb with genets that consist of dimorphic ramets. Flowering ramets have 2–3 leaves with a terminal inflorescence consisting of 4–35 perfect flowers, while vegetative ramets have only 1 leaf. Mayflower is self-incompatible (Worthen and Stiles 1986; TD Lee unpublished data) and is insect pollinated (Thaler and Plowright 1980), and has been shown to be pollen limited in New Jersey populations (Worthen and Stiles 1988). Fruits contain 1–4 seeds. Individual ramets have been reported to provide "virtually all" of the resources needed to mature their own fruits and seeds and experimentally defoliated ramets were found to be subsidized by other ramets (Silva 1978 cited in Pitelka and Ashmun 1985). In the fall, ramets "die back," leaving an overwintering bud that will become next year's ramet. At this point it is possible to determine by touch whether the bud is vegetative or flowering. The status of this bud appears to be determined much earlier, and flower primordia may be visible under a microscope as soon as May (Kana 1982). With the "die back" of the ramets, an abscission scar is left. The scar is distinct for vegetative and flowering ramets, and it is possible to determine the age of the ramet and whether the ramet has been flowering or vegetative for each of its previous years (ramet history) (Silva et al. 1982; Fig. 1).

A field experiment was used to address the following questions about mayflower. Is there a difference in the current sexual reproduction of ramets between two years? Is mayflower pollen limited in local populations? Is there physiological integration such that ramets connected to the genet are more successful than ramets severed from the genet?

Methods

The experiment was located within a mixed canopy forest dominated by white pine (Pinus strobus), various hardwoods, and eastern hemlock (Tsuga canadensis) in southeastern New Hampshire. In the spring of 1994, seven experimental blocks were selected based on the presence of mayflower. Blocks were separated by at least 30 m and were intended to limit the potential of a genet bias and/or a location effect. Buds within each block were felt by hand and twenty buds identified as flowering buds were randomly assigned to each of four treatments. Two of the treatments consisted of severing the rhizome on either side of the ramet (Fig. 1). This process involved partially excavating the rhizome, severing the rhizome with a scalpel such that each ramet

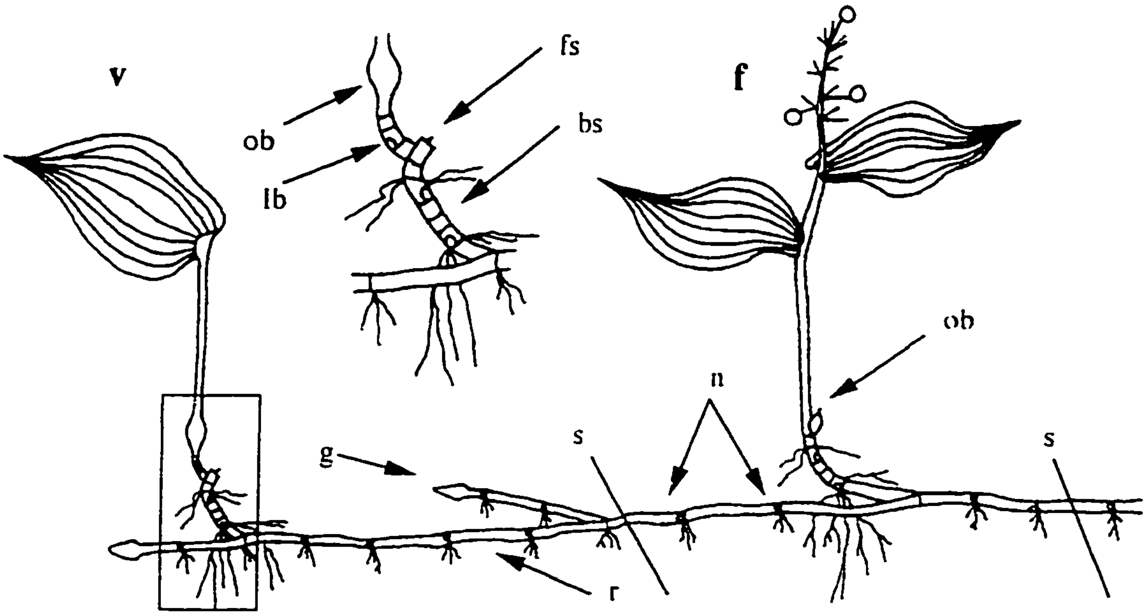

Fig. 1 Portion of a mayflower genet consisting of a vegetative ramet (v) and a flowering ramet (f) that has matured three fruits. The location of the overwintering bud (ob) is noted. A new rhizome (g) develops from a lateral bud located at a node (n) and at some point in the rhizome's development it will turn upward and become a new ramet. Flowering ramets in the severing treatments had their rhizomes (r) severed so as to include two nodes (n) on either side of the ramet. The vegetative ramet (v) is five years old and the flowering ramet scar (fs) shows that this ramet flowered in its third year. The location of a lateral bud (lb) and bud scales (bs) are also noted.

contained two nodes on either side, and then re-covering the rhizome with soil. Ramets within the other two treatments were partially excavated and treated similarly except that the rhizome was not severed.

In May, when the ramets began to flower, all flowers in one of the "severed" treatments and in one of the "intact" treatments received an overabundance of exogenous pollen each day for the life of the flowers. Pollen was collected immediately prior to use from 10–20 flowering ramets not more than 20 m distant and applied to the treatment ramets with a toothpick. The other severed treatment and intact treatment were not manipulated and thus presumably received natural levels of pollination. The fates of fruits and seeds were followed throughout the season and the number matured was noted for each ramet. After a final number of fruits and seeds was determined in late August, the ramets were excavated and the history of each ramet was determined.

In the spring of 1995, a similar experiment was undertaken in the same mixed canopy forest except that three experimental blocks were used instead of seven and the number of replicates per treatment was increased from five to ten. Blocks were separated by at least 30 m.

The number of seeds that an individual matures is an important aspect of fitness in that each seed may equal a distinct individual in the future. However, the number of fruits that an individual matures may also be an important aspect of fitness since fruits are the method by which many seeds are dispersed (Lee 1988). If fruits are dispersed whole, then a plant that matures few-seeded fruits may have a greater fitness than a plant with a similar number of seeds that matures many-seeded fruits (Casper and Wiens 1981). Alternatively, if many-seeded fruits are selectively eaten by animal dispersers, then a plant maturing many-seeded fruits may have a greater fitness than a plant with a similar number of seeds that matures few-seeded fruits (Lee 1988).

A multiple analysis of variance (MANOVA) was used to determine differences in both seed number and fruit number. A MANOVA was preferred over two separate analyses of variance (ANOVA) since performing two ANOVAs would have required the assumption that seed number and fruit number had a zero correlation or that this correlation was not of interest (Bray and Maxwell 1985). The MANOVA was able to evaluate seed number and fruit number simultaneously and also considered any potential correlation between the two variables (Bray and Maxwell 1985). The MANOVA created a new "variate" by linearly combining the two dependent variables. The Pillai trace statistic was used to determine significance since it is robust to possible violations in the assumptions of the analysis (Scheiner 1993; Bray and Maxwell 1985). If the Pillai statistic yielded significance then the canonical correlation squared indicated how much variation in the new variate was explained by the variation in the independent variable.

The analysis was intended to include a blocking term, but in 1994 five replicates per block were not enough to offset losses of individuals within different treatments among blocks. Thus, few blocks contained the complete experiment. Variation was therefore not partitioned out for block{year} but was instead added to the error term (unexplained variation). Three factors were included in the analysis: time (1994 vs. 1995), pollination (overpollinated vs. open pollinated), and severing (severed vs. intact). Each of these factors was fixed, and the second and third order interactions were included in the analysis (Table 1).

Four pairwise comparisons were used to answer specifically the experimental questions. In order to preserve the experimental α at 0.05, a Bonferroni adjusted α of 0.0125 (0.05 / 4 comparisons) was used as recommended by Winer et al. (1991).

Results

Eighteen ramets either were lost or were not able to be aged accurately. The 242 remaining ramets used in this experiment were found to be, on average, 5.78 years old (S.D.=2.71 years; Fig. 2). One hundred ninety-four of these ramets were found to have flowered once in their lifetimes, thirty-eight were found to have flowered twice, and 9 were found to have flowered three times. Of the ramets that flowered once, the mean number of years prior to flowering was 3.97 years (S.D.=2.03 years; Fig. 3) and no ramet had flowered in its first year. Of the 48 ramets that flowered more than once, the average number of years between flowering events was 2.71 years (S.D.=1.50 years; Fig. 4), and no ramet was found to have flowered two years consecutively. There was a positive relationship between the age of the ramets and the number of times that they flowered during their lifetime ($p<<<0.001$, adj. $r^2=0.422$, $n=242$).

The MANOVA of seed and fruit number (Table 1) showed that there was a significant difference in the current sexual reproduction of ramets in 1994 and 1995 (Pillai trace=0.214, $F_{2, 170, 0.05}=23.02$, $p<<<0.001$). The year of the experiment explained 21.4% of the variation in the new variate (a linear combination of both seed number and fruit number). Ramets in 1994 matured comparable numbers of seeds as those in 1995 (univariate $F_{1, 163, 0.05}=0.015$, $p=0.902$). However, there was a difference in the number of fruits matured (univariate $F_{1, 163, 0.05}=6.531$, $p=0.012$) and, thus, how the seeds were distributed among fruits differed as well. Rameets in 1994 matured fewer fruits with a

Table 1 Results of a multiple analysis of variance (MANOVA) performed on the number of fruits and seeds matured

Source	Pillai Trace	Multivariate F	p-value	Canonical Correlation
YEAR	0.214	$F_{2,170,0.05}=23.02$	$p<<0.001$	0.463
POLLEN	0.008	$F_{2,170,0.05}=0.709$	NS	
SEVERING	0.076	$F_{2,170,0.05}=6.994$	$p<0.005$	0.276
YEAR*POLLEN	0.001	$F_{2,170,0.05}=0.068$	NS	
YEAR*SEVERING	0.026	$F_{2,170,0.05}=0.104$	NS	
POLLEN* SEVERING	0.059	$F_{2,170,0.05}=5.344$	$p<0.01$	0.243
YEAR*POLLEN* SEVERING	0.021	$F_{2,170,0.05}=1.789$	NS	

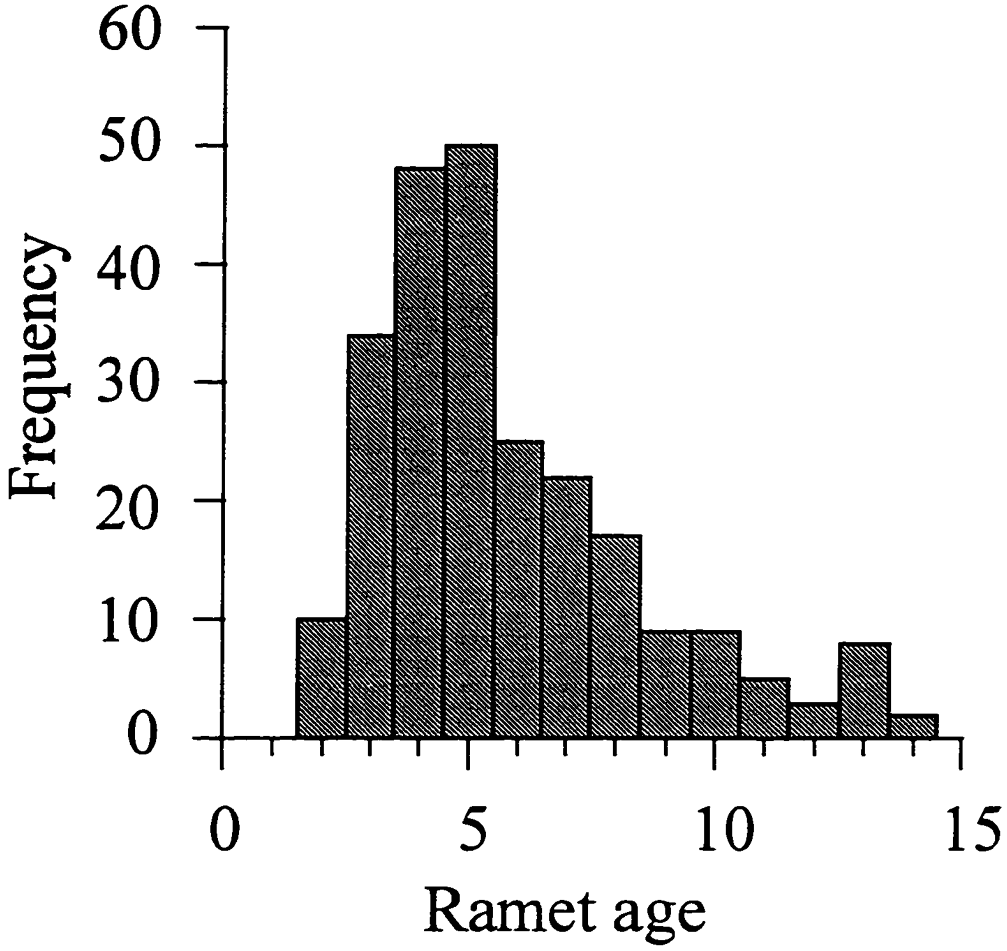

Figure 2. Histogram of ages of ramets used in this experiment (n=242, mean=5.78, SD=2.71).

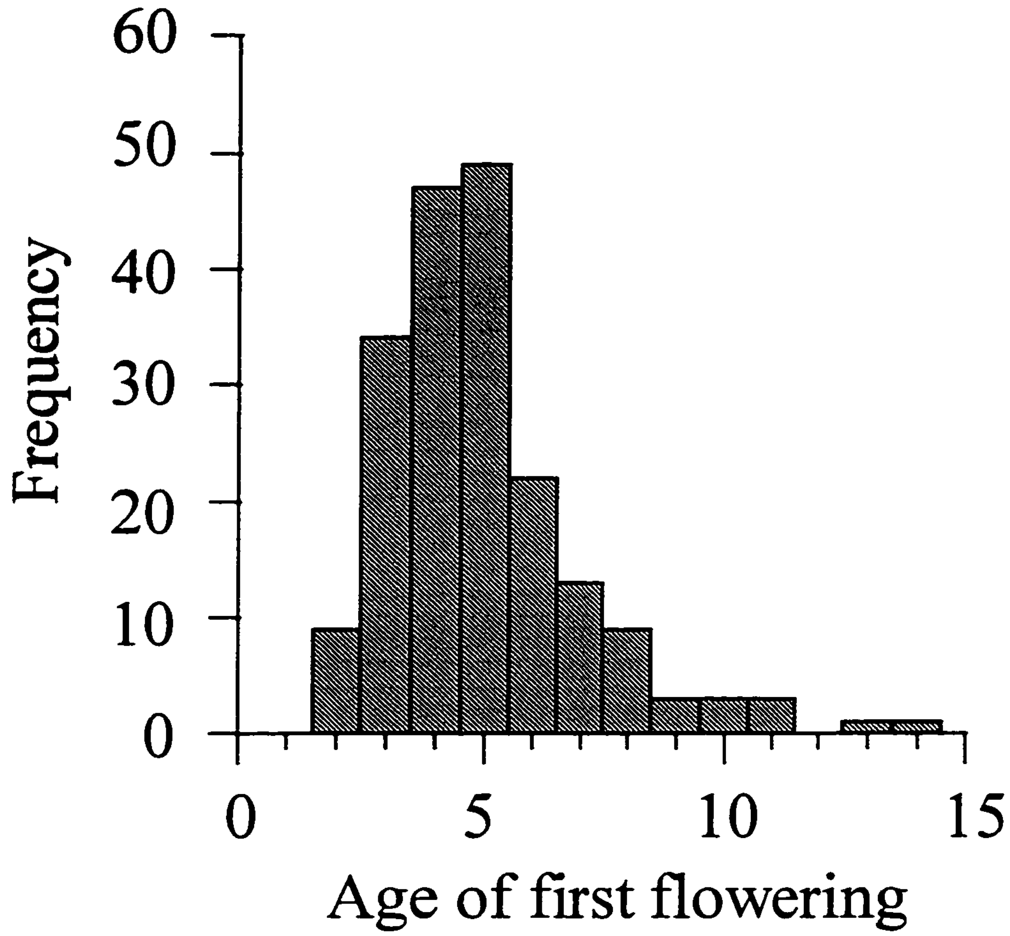

Figure 3. Histogram of ages at which the experimental ramets first flowered (n=194, mean=3.97, SD=2.03).

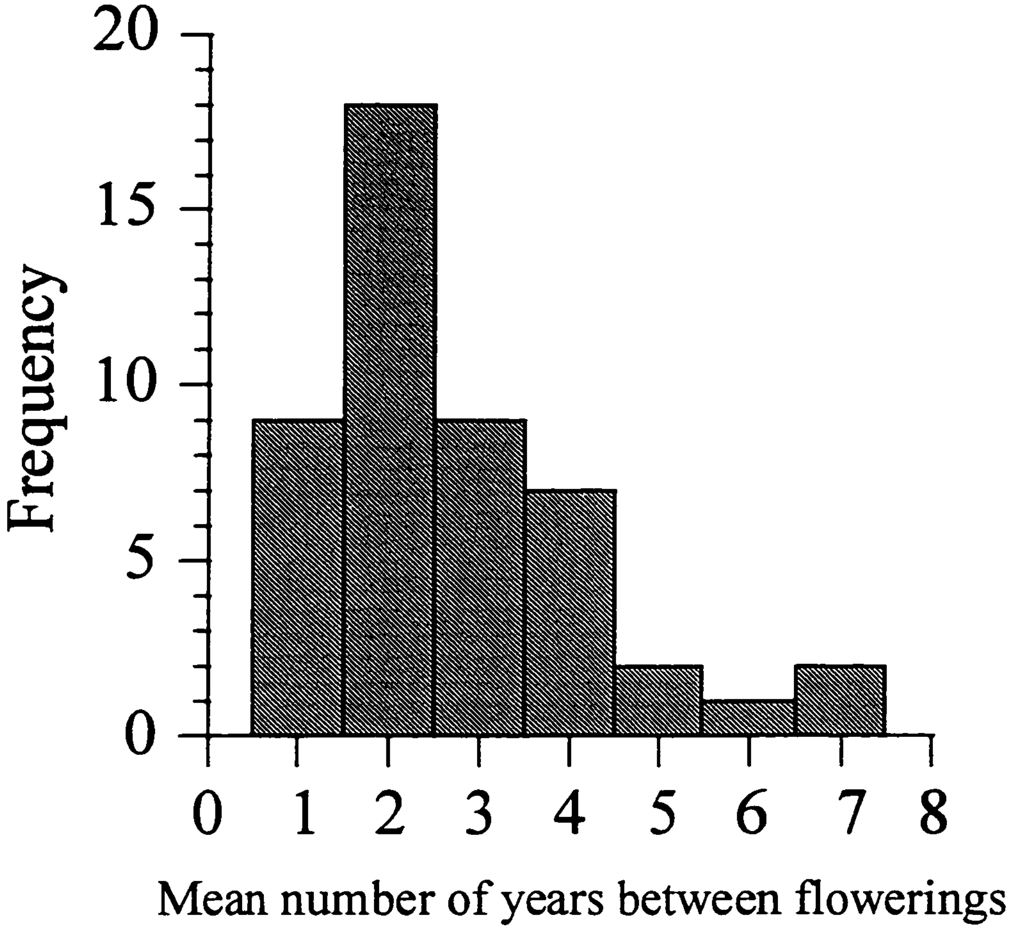

Figure 4. Histogram of the mean number of years between flowerings (n=48, mean= 2.71, SD=1.50). This histogram includes only ramets that flowered more than once during their lifetime.

greater number of seeds/fruit while ramets in 1995 matured more fruits with fewer seeds/fruit (Fig. 5; Table 2).

In order to test for pollen limitation, the overpollinated, intact treatment was compared with the open pollinated, intact treatment. There was a significant difference between the two treatments (Pillai trace=0.063, $F_{2, 162, 0.05}$=6.165, p=0.003). The addition of pollen explained 7.7% of the variation in the new variate (a linear combination of both seed number and fruit number). The ramets of the overpollinated, intact treatment matured more seeds (univariate $F_{1, 163, 0.05}$=10.258, p=0.002) and more fruits (univariate $F_{1, 163, 0.05}$=5.470, p=0.002) than the ramets of the open pollinated, intact treatment. The number of seeds/fruit did not differ between treatments (Fig. 6; Table 2).

In order to test for physiological integration, two comparisons were made. The open pollinated, intact treatment was compared with the open pollinated, severed treatment and there was no difference between the two (Pillai trace=0.001, $F_{2, 162, 0.05}$=0.002, p=0.998). The overpollinated, intact treatment was compared to the overpollinated, severed treatment and, here, there was a significant treatment effect (Pillai trace=0.132, $F_{2, 162, 0.05}$=12.371, p<<<0.001). Severing explained 13.2% of the variation in the new variate (a linear combination of both seed number and fruit number). The overpollinated, intact ramets matured more seeds (univariate $F_{1, 163, 0.05}$=19.787, p<<<0.001), fruits (univariate $F_{1, 163, 0.05}$=9.969, p=0.002), and seeds/fruit than did the ramets of the overpollinated, severed treatments (Fig. 7; Table 2).

The overpollinated, severed treatment was compared to the open pollinated, severed treatment and there was no significant difference between the two (Pillai trace=0.012, $F_{2, 170, 0.05}$=0.989 , p=0.374; Table 2).

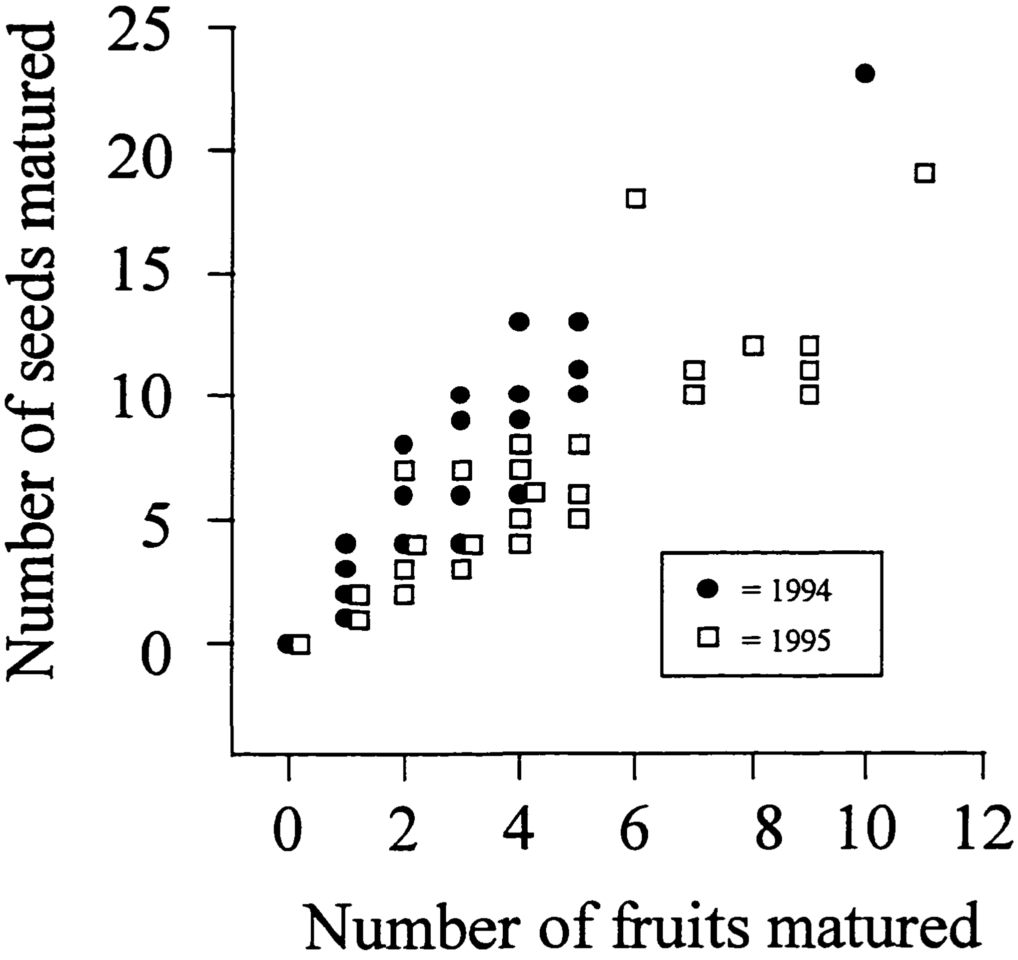

Figure 5. Relationship between the number of seeds matured and the number of fruits matured for ramets from 1994 and 1995.

Table 2 The mean number and standard deviation (S.D.) of fruits and seeds matured per ramet in both years of the experiment and in each of the four treatments. Small case letters denote treatment means that are not significantly different. Comparisons are made separately for fruit number and seed number. Comparisons between years are also made separately from comparisons among the four treatments.

	FRUIT		SEED	
	MEAN	S.D.	MEAN	S.D.
1994	1.371 a	1.979	3.300 a	4.807
1995	2.147 b	2.391	3.009 a	3.765
overpollinated, intact	2.694 a	2.785	5.163 a	5.728
overpollinated, severed	1.244 b	1.694	1.622 b	2.300
open pollinated, intact	1.770 c	2.101	2.904 c	3.604
open pollinated, severed	1.515 b,c	2.048	2.485 b,c	3.251

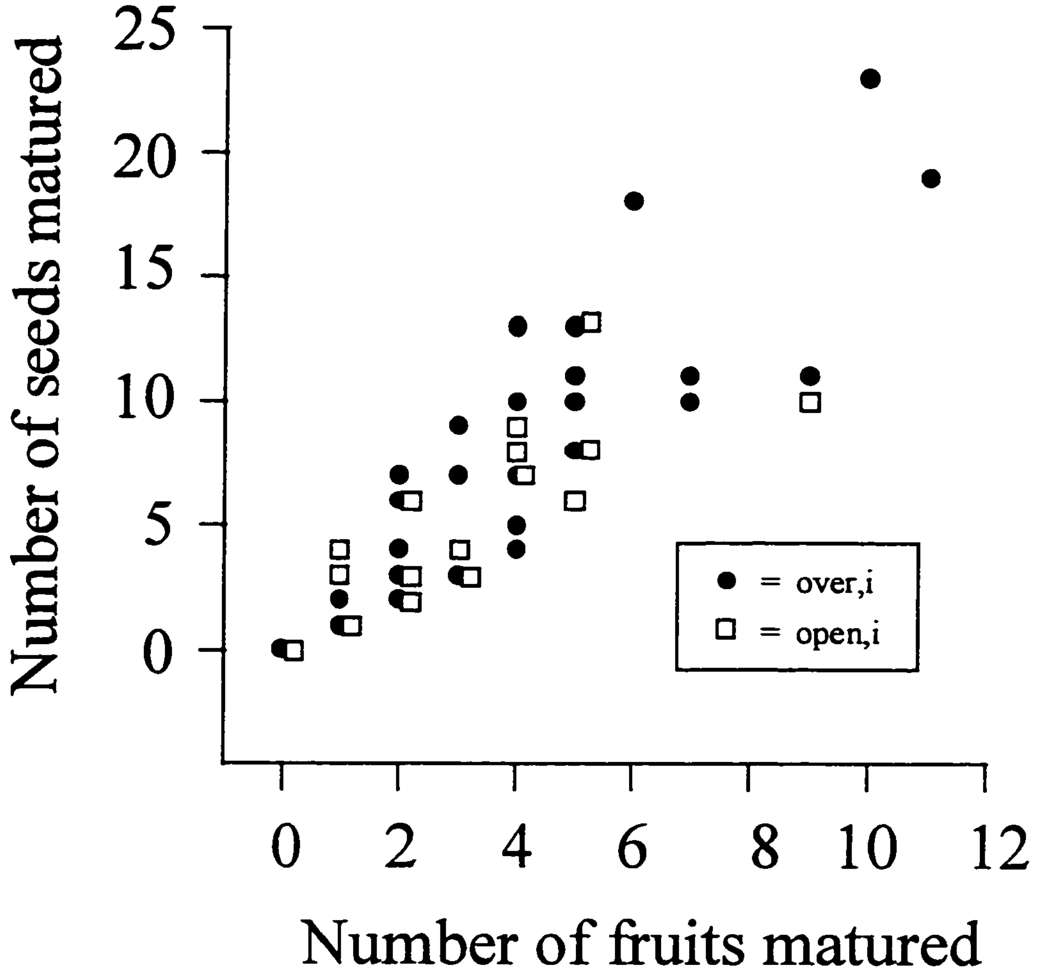

Figure 6. Relationship between the number of seeds matured and the number of fruits matured for ramets from the overpollinated, intact (over,i) and the open pollinated, intact (open,i) treatments.

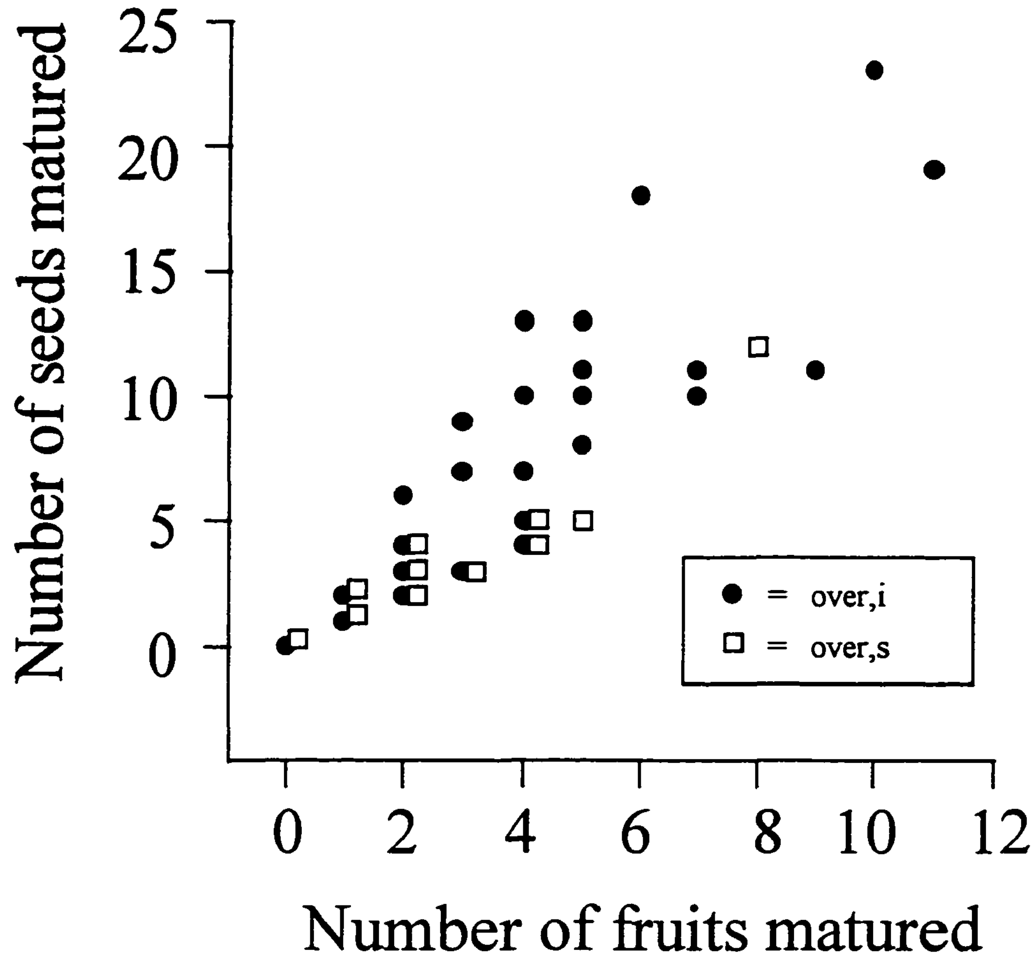

Figure 7. Relationship between the number of seeds matured and the number of fruits matured for ramets from the overpollinated, intact (over,i) and the overpollinated, severed (over,s) treatments.

Discussion

There was a significant difference in the number of fruits matured and the number of seeds/fruit between years that is reflected in all treatments. While the number of seeds matured did not differ between years, the way in which seeds were distributed among fruits did differ. Ramets in 1994 tended to mature fewer fruits with more seeds per fruit, and ramets in 1995 tended to mature more fruits with fewer seeds/fruit. It is likely that some form of resource limitation and not pollen limitation was responsible for the difference in fruit number. It could be that fruits in 1994 contained a greater number of seeds/fruit on average because individual ramets selectively aborted fewer-seeded fruits (Stephenson 1981). Ramets in 1995 may not have experienced resource limitation to the same degree; therefore, the fruits with fewer seeds were retained.

Mayflower ramets have been demonstrated to be pollen limited in New Jersey populations (Worthen and Stiles 1988). In both 1994 and 1995 mayflower ramets were shown to be pollen limited in local New Hampshire populations as well. Although seed and fruit number increased with pollen addition, the proportion of variation in current sexual reproduction explained was only 7.7%. Many ramets that received an overabundance of exogenous pollen matured comparable numbers of seeds as the open pollinated ramets, and in fact, eleven of the overpollinated, intact ramets matured zero seeds. The low number of seeds and fruits in the open pollinated, intact ramets is attributed to a lack of pollen. However, a lack of compatible pollen could have produced this result as well. Handel (1985) has demonstrated that older, more central ramets of Carex platyphylla and Trifolium repens are more likely to receive self pollen than younger, more distally located ramets. This effect is possible for mayflower, although not as likely, since ramets spend the bulk of their time as vegetative ramets and, therefore, there is a low probability that a flowering ramet's nearest-neighbor ramet on the same genet is also flowering.

Ramets that were open pollinated matured comparable numbers of seeds, fruits, and seeds/fruit whether their rhizomes were severed or not. This suggests that at natural levels of pollination the ramets had adequate resources to support the seeds and fruits that resulted from pollination. Ramets may have drawn on photosynthates stored in "local" rhizomes or on their own photosynthates that were being produced currently. Ramets and the local rhizome appeared to have had enough root mass to provide sufficient water to support current sexual reproduction. However, ramets that received an overabundance of pollen differed with regard to the number of seeds, fruits, and seeds/fruit matured, depending on whether the rhizome was severed or not. Thus, it appears that the genet may play a role in increasing a ramet's realized sexual reproduction when pollen is not limiting. It is not clear whether other ramets supplemented the experimental ramets or if the experimental ramets drew on a larger resource pool composed of resources stored in the rhizome by other ramets and by the experimental ramet in the past. It may also be that a larger root mass was needed to mature more seeds and fruits and that a larger rhizome meant a larger root mass which would have been unavailable to the severed treatment. With regard to current sexual reproduction, individual ramets may be more or less independent at natural levels of pollination, but as the level of pollination increases ramets require support, in some form, from the genet.

Seed production may be limited by a number of factors including pollen availability (Widén 1992; Johnston 1991; Bierzychudek 1981) and resource availability

(Stephenson 1981). Pollen limitation and resource limitation may act together to influence seed production (Haig and Westoby 1988) or may act on different aspects of sexual reproduction such as flower and seed production (Campbell and Halama 1993). Plants demonstrated to be pollen limited in one year may ultimately be resource limited (Ehrlén and Eriksson 1995; Bierzychudek 1981). This may be the case for mayflower ramets. Individual ramets do not flower two years consecutively; instead there is a period of years that separates the flowering events. Time may be required for local resource accumulation and storage. If the probability of flowering increases with a larger resource store, then a greater allocation of resources to current sexual reproduction may delay subsequent flowerings, or even reduce the success of future sexual reproduction.

While mayflower ramets were pollen limited during the years of this experiment, it is not possible to evaluate the extent to which pollen limitation and resource limitation may interact, since resources were not manipulated directly. Rhizomes were either severed to prevent the potential for physiological integration or left intact to allow for the possibility of physiological integration. Resources external to the genet were not manipulated; instead the distribution of resources internal to the genet was influenced. Ramets that were overpollinated and whose rhizome was left intact were able to mature more seeds because they received an overabundance of pollen and were presumably able to generate a strong sink that capitalized on resources available in another part of the genet (Watson and Casper 1984). These resources would be unavailable to ramets whose rhizomes were severed. The concepts of pollen limitation and resource limitation need to be placed within the context of the entire genet. It is possible that a ramet, pollen limited in a given year, is within a genet that is pollen limited or even resource limited. It is also possible that a ramet, resource limited in a given year, is within a genet that is pollen limited or resource limited. Future research should concentrate on what effect, if any, current sexual reproduction in one ramet has on both future reproduction in the same ramet as well as on the current and future reproduction of ramets within the same genet.

CHAPTER II

THE SUMMER OF 1994 REVISITED: FLOWERS AND RHIZOMES

Abstract

Canada mayflower (Maianthemum canadense var. canadense Desf.) was the subject of a field experiment to determine if rhizome severing 1) reduced the number of flowers brought to anthesis by ramets, 2) delayed flowering, or 3) reduced the length of time that flowers were receptive to pollen. An experiment was also undertaken to determine if the number of rhizomes initiated by flowering ramets was related to whether their flowers were overpollinated, open pollinated, or bagged, or whether their rhizomes were severed or left intact. Ramets whose rhizomes were severed brought, on average, 3.2 fewer flowers to anthesis than ramets whose rhizomes were left intact. Ramets whose rhizomes were severed did not appear to delay flowering or reduce the length of time that flowers were receptive. Rhizome severing resulted in an increase in the number of rhizomes initiated. However, the number of rhizomes initiated was independent of pollination level (open pollinated, overpollinated, bagged, and vegetative).

Introduction

In Chapter I, the number of fruits and seeds matured by overpollinated Canada mayflower (Maianthemum canadense var. canadense Desf.) ramets was reduced in ramets whose rhizomes were severed compared to those whose rhizomes were left intact. It was concluded that ramets with intact rhizomes did not experience resource limitation to the same degree as ramets whose rhizomes were severed. This chapter investigates the effects of severing and overpollination on other aspects of sexual and clonal reproduction, specifically the number of flowers brought to anthesis, the timing of anthesis, the length of time that flowers are receptive, and the number of rhizomes that a ramet initiated.

For some insect-pollinated plants, infloresence size is positively correlated with the number of fruits matured by the plant (Willson and Bertin 1979). For mayflower, the number of flower primordia are determined early in the year, prior to the growth of the flowering ramet (Kana 1985). The number of flower primordia may exceed the number of flowers actually brought to anthesis. This may represent "bet hedging" and occurs in other insect pollinated plants (Stephenson 1981).

For mayflower that were overpollinated, rhizome severing reduced seed and fruit maturation. If rhizome severing affected resource levels before flowers expanded, then the number of flowers actually brought to anthesis in these ramets may have been lower than the number of flowers brought to anthesis by ramets whose rhizomes were left intact. The timing of flowering may have been different as well, with ramets whose rhizomes were severed delaying flowering or shortening the length of time that individual flowers were receptive to pollination.

Clonal plants must allocate limited resources to growth, sexual reproduction, and ramet production. It is widely accepted that allocation of resources to one form of reproduction should come at the expense of the other form of reproduction (Abrahamson 1975, Williams 1975). Thus, if severing increases the allocation of resources to ramet production (rhizome initiation), then sexual reproduction may be reduced.

The following questions were addressed in an experimental study with mayflower: Did ramets separated from the genet via rhizome severing bring fewer flowers to anthesis than ramets left connected to the genet? Was a reduction in the number of flowers brought to anthesis associated with a reduction in the number of fruits matured? Did ramets severed from the genet delay flowering when compared to ramets that remain connected to the genet? Was there a relationship between the number of rhizomes initiated and the number of seeds matured by ramets?

Methods

The experiment was located within a mixed canopy forest dominated by white pine (Pinus strobus), hardwoods, and eastern hemlock (Tsuga canadensis) in the University of New Hampshire woodlands, Durham, NH. In the spring of 1994, seven experimental blocks were selected based on the presence of mayflower. These blocks were separated by at least 30 m and were intended to limit the potential of a genet bias, a location effect, or both. Thirty buds within each block identified as flowering buds, based on size and shape, were randomly assigned to each of six treatments. Three of the treatments consisted of severing the rhizome on either side of the ramet (Fig. 1). This process involved partially excavating the rhizome, severing the rhizome with a scalpel such that each ramet contained two nodes on either side, and then re-covering the rhizome with soil. Ramets within the other three treatments were partially excavated and treated similarly except that the rhizome was not severed. Ten buds within each location identified as vegetative were selected and randomly assigned to each of two treatments. One treatment consisted of severing the rhizome in the same way as were rhizomes of flowering buds. Rhizomes in the other treatment were not severed.

In May, when the ramets began to flower, all flowers in one of the "severed" treatments and in one of the "intact" treatments received an overabundance of exogenous pollen each day for the life of the flowers. Pollen was collected immediately prior to use from 10–20 flowering ramets not more than 20 m away and applied to the treatment ramets with a toothpick. All of the flowers in one of the severed and one of the intact treatments were open pollinated and thus presumably received natural levels of pollination. All of the flowers in the final severed and intact treatments were covered with a dialysis tubing bag for the life of the flowers. The tubing prevented insect pollination but permitted moisture and gas exchange. The two overpollinated treatments and the two open-pollinated treatments contained the same ramets discussed in Chapter I.

The number of flowers that each ramet brought to anthesis was determined. The number of flowers that were receptive each day for ramets within each of the two overpollinated treatments was also determined. In mid-September, after fruiting had finished, all ramets (flowering and vegetative) were excavated and the number of rhizomes that each ramet initiated was determined. Rhizomes were counted as initiated if there was new rhizome growth from the two nodes on either side of the ramet.

A two-way analysis of variance (ANOVA) was used to determine if there was a difference in the number of flowers brought to anthesis among treatments. Two factors were included in the analysis: pollination (overpollinated, open pollinated, and bagged) and severing (severed vs. intact). Each of the factors was fixed and the interaction term was included in the analysis.

The number of fruits matured by the open-pollinated, intact ramets was regressed on the number of flowers brought to anthesis by these ramets to determine in there was a relationship.

The number of flowers at anthesis each day was determined for the overpollinated, severed and the overpollinated, intact ramets. A histogram was produced for each treatment based on these values. The median and mean date of flowering was calculated for each treatment. No analysis was performed on these data since the number of flowers at anthesis on a particular day was not independent of the number of flowers at anthesis on the previous day. The date of first flowering was determined for ramets in the overpollinated, severed and the overpollinated, intact treatments. A non-parametric ANOVA was used to determine if the date of first flowering differed between treatments.

A two-way ANOVA was used to determine differences in the number of rhizomes initiated among ramet treatments. Two factors were included in the analysis: pollination (overpollinated, open pollinated, bagged, and vegetative) and severing (severed vs. intact). Each factor was fixed and the interaction was included in the analysis.

Results

Flowering ramets whose rhizomes were severed brought significantly fewer flowers to anthesis than flowering ramets that had their rhizomes left intact ($F_{1,117,0.05}=5.324$, $p<0.05$, $r^2=0.104$; Table 3, Fig. 8). Neither the pollination ($F_{2,117,0.005}=0.528$, $p=0.568$) nor the pollination*severing interaction ($F_{2,117,0.05}=2.742$, $p=0.069$) was significant.

Table 3. The mean number and standard deviation (SD) of flowers brought to anthesis for each of the six flowering ramet treatments.

Treatment	mean	SD
overpollinated, intact	20.7	6.67
open pollinated, intact	16.3	6.12
bagged, intact	18.0	7.12
overpollinated, severed	14.8	6.98
open pollinated, severed	16.3	7.54
bagged, severed	14.3	7.77

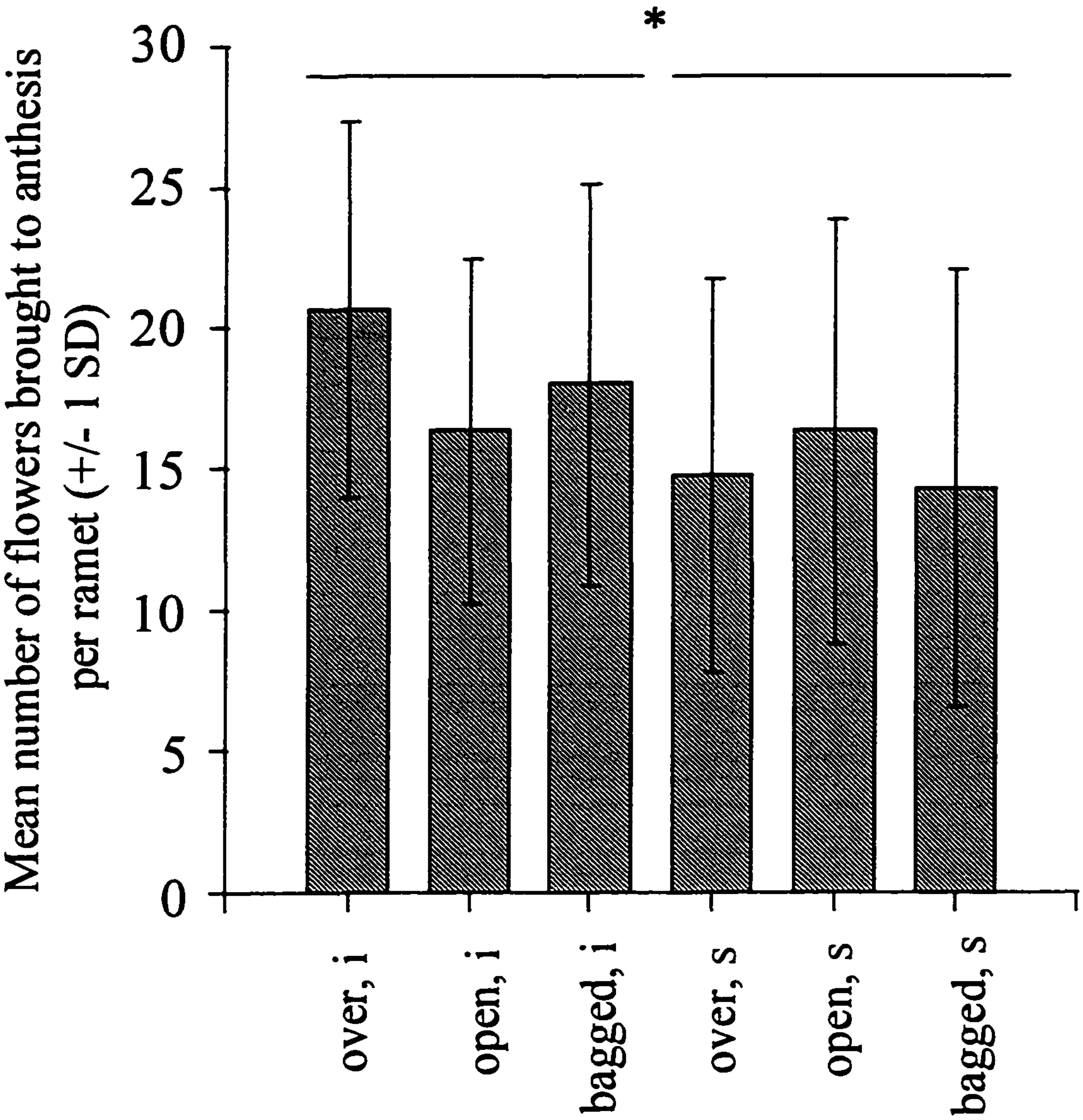

Figure 8. The mean number and standard deviation (SD) of flowers brought to anthesis for each of the three intact (i) ramet treatments and the three severed (s) ramet treatments.

The number of fruits matured was not related to the number of flowers matured by the open-pollinated, intact ramets ($p=0.822$, $n=21$; Fig. 9).

The median date of flowering in the overpollinated, intact treatment was May 28 and the mean date of flowering was May 27. The median date of flowering in the overpollinated, severed treatment was May 29 and the mean date of flowering was May 28 (Fig. 10). The date of first flowering for ramets in each of the overpollinated treatments did not differ (Mann Whitney U test statistic=160, $p=0.533$).

Ramets whose rhizomes were severed initiated more rhizomes than ramets whose rhizomes were left intact ($F_{1,191,0.05}=16.489$, $p<0.0001$, $r^2=0.122$; Fig. 11). There was no difference in the number of rhizomes initiated by ramets attributable to the pollination factor ($F_{3,191,0.05}=0.838$, $p=0.475$) or the interaction of pollination and severing ($F_{3,191,0.05}=2.419$, $p=0.068$).

Discussion

Flowering ramets with intact rhizomes have been shown to mature more seeds and fruits than flowering ramets with severed rhizomes when pollen is not limiting (Chapter I). The data from this experiment suggest that flowering ramets whose rhizomes are left intact are also able to bring more flowers to anthesis than ramets whose rhizomes were severed. The average difference in the number of flowers brought to anthesis was 3.2 flowers and rhizome severing explained only 10.4% of the variation in the number of flowers brought to anthesis. Since the flower primordia were produced much earlier than the experimental manipulations, it is likely that rhizome severing induced flower abortion. In fact, flower abortion was observed in the experimental ramets (Ganger, personal

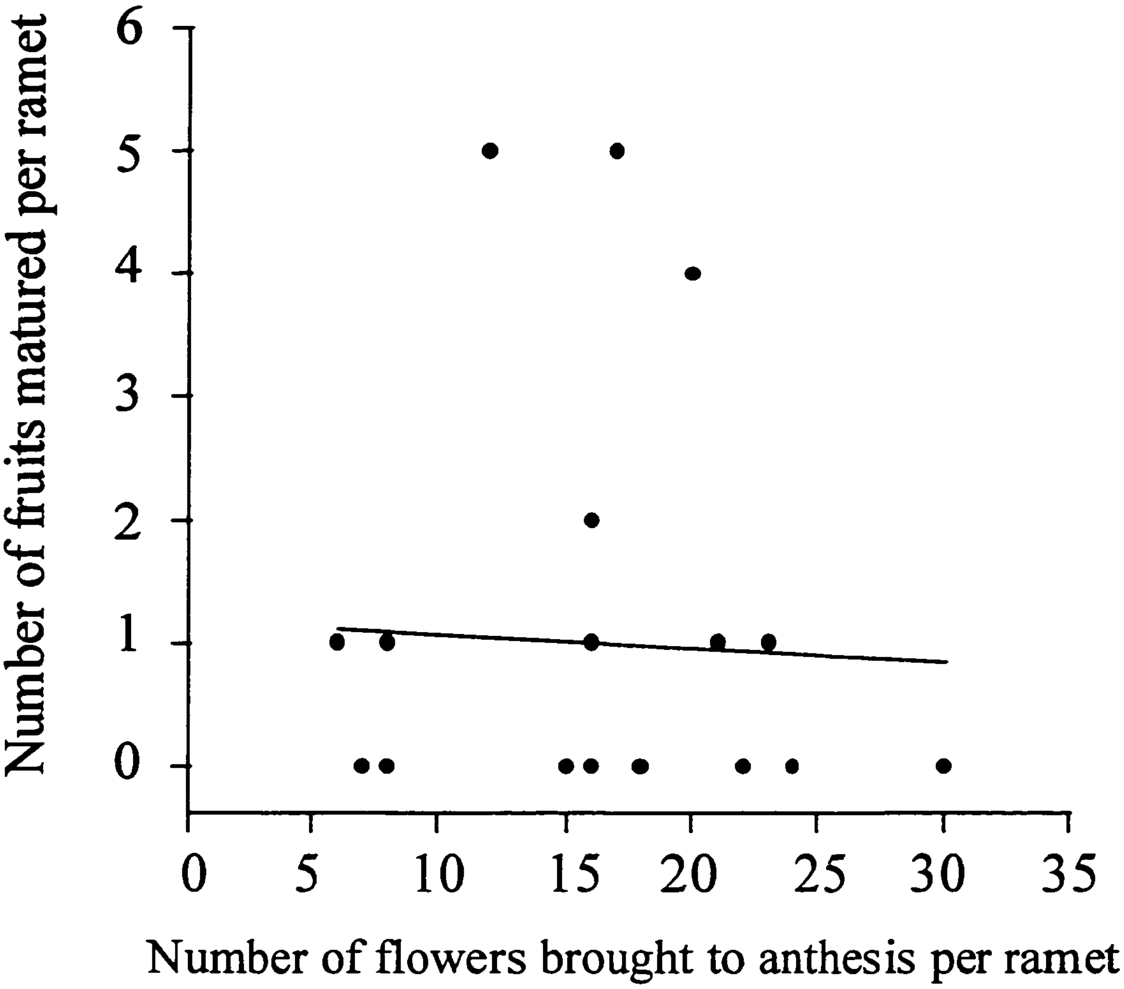

Figure 9. The relationship between the number of flowers brought to anthesis and the number of fruits matured by each open-pollinated, intact ramet.

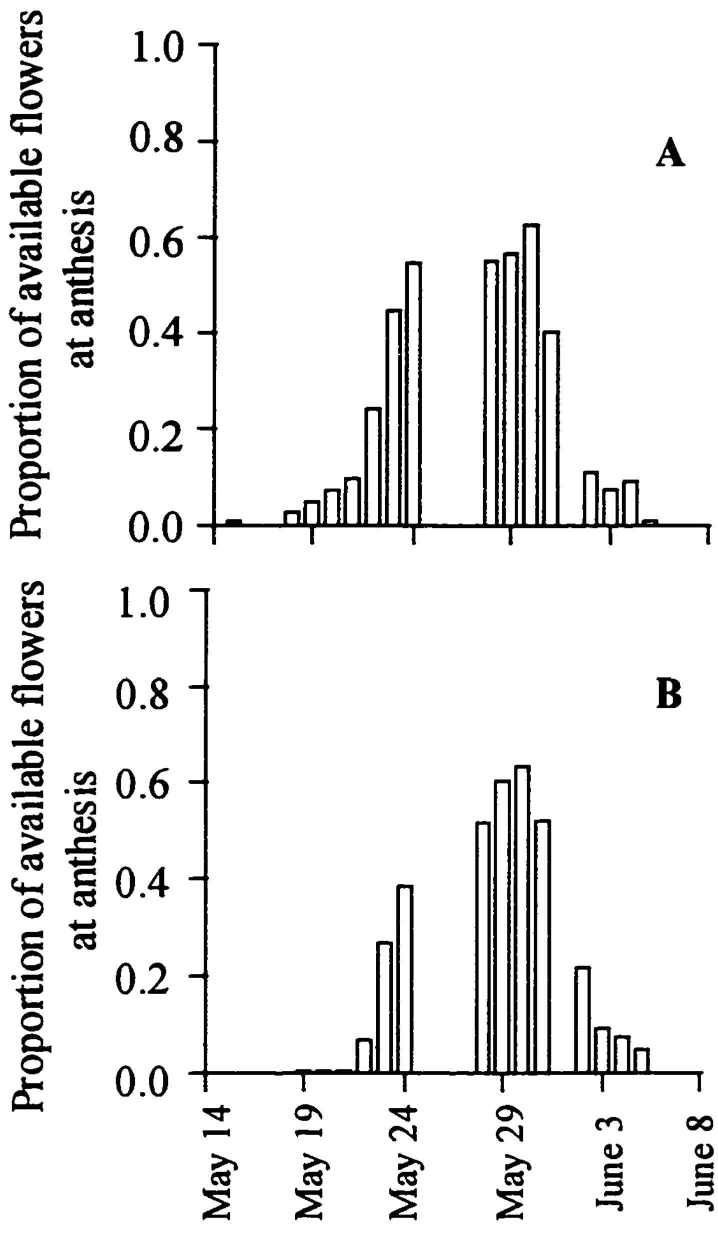

Figure 10. The distribution of the number of flowers at anthesis for **A** overpollinated, intact ramets and **B** overpollinated, severed ramets. No data are plotted for May 16, May17, May 25, May 26, May 27, or June1. Rain on these days prevented the determination of the number of flowers at anthesis. The data presented here are not the same data that are statistically tested.

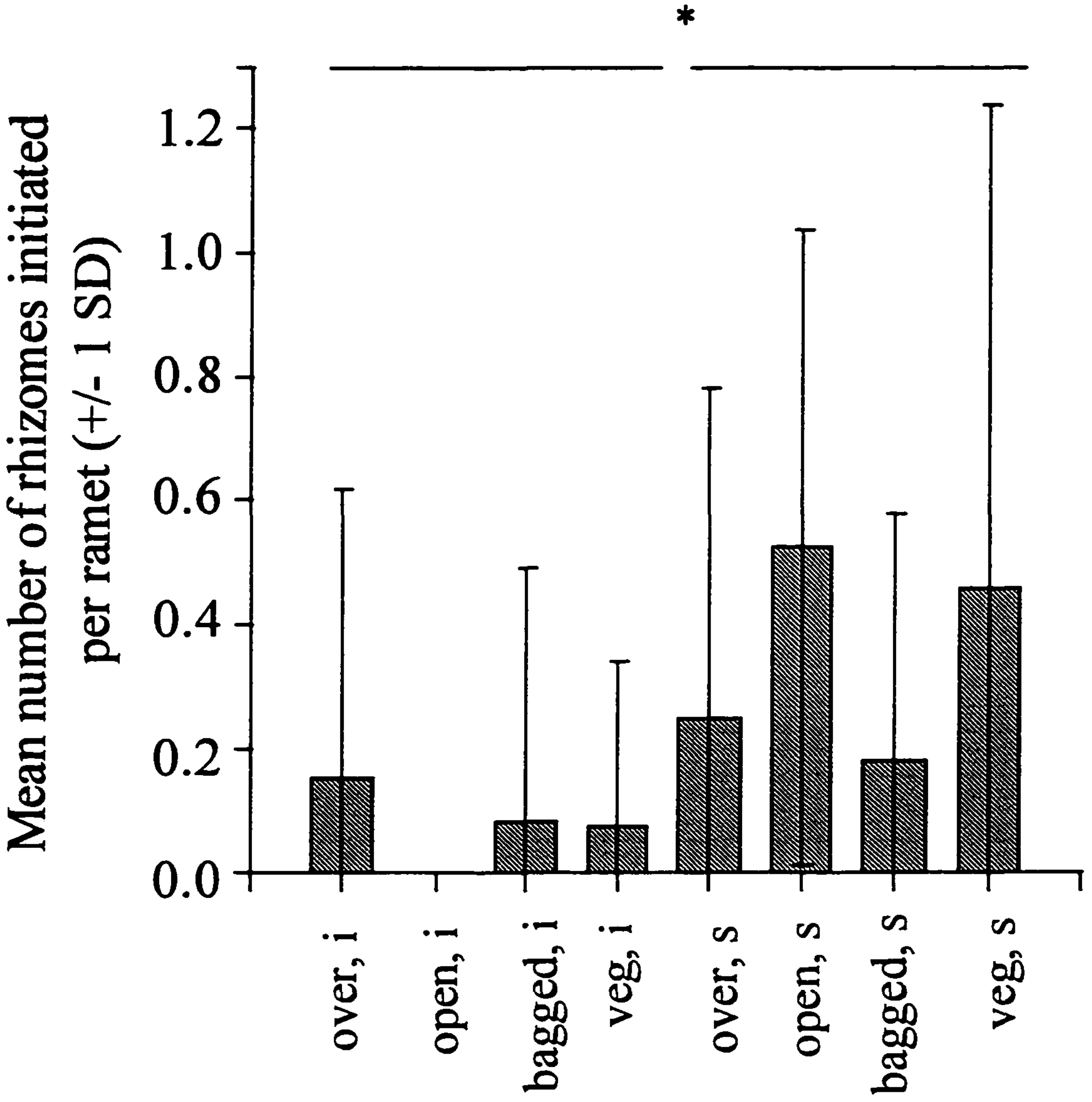

Figure 11. The mean number and standard deviation (SD) of mayflower rhizomes initiated by each of the four intact (i) treatments and the four severed (s) treatments.

oberservation). Ramets whose rhizomes were severed would not have been able to draw resources from other parts of the genet while ramets whose rhizomes were left intact would have. The large amount of variation in the number of flowers brought to anthesis not explained by rhizome severing (89.6%) may have been due to the resource state of the ramets prior to the manipulations. For example, some ramets that produced very few flower primordia may have been assigned to the intact treatment and some ramets that produced many flower primordia, due to being situated in a high resource area, may have been assigned to the severed treatment.

It does not appear that a reduction in the number of flowers brought to anthesis reduced the number of fruits matured. The proportion of flowers maturing fruit in mayflower was almost always much less than one and therefore the loss of a few flowers (average of 3.2) should not have constrained fruit and seed production much if at all.

It is possible that ramets that were resource limited early in the season may have delayed bringing flowers to anthesis or remained in this stage (receptive) for a shorter period of time (Primack 1985) and this in turn may have been responsible for the reduction in fruits and seeds observed in ramets whose rhizomes were severed. Ramets whose rhizomes were severed may have indeed been resource limited early in the season, as evidenced by the decreased number of flowers that they brought to anthesis. While there was a difference in the number of flowers receptive on a given day between overpollinated ramets whose rhizomes were severed or left intact, there did not appear to be a difference between them in either the median or mean date of flowering. There was no difference in the date of first flowering. Any resource limitation experienced by the ramets whose rhizomes were severed probably did not affect the flowering phenology of these ramets.

Ramets whose rhizomes were severed initiated more rhizomes than ramets whose rhizomes were left intact. Whether the ramets were overpollinated, open pollinated, bagged, or vegetative did not have an effect on the number of rhizomes initiated. The increased initiation of rhizomes may have been due to the release of lateral buds at the nodes from inhibition (Phillips 1975, Cook 1985). The relationship between apical dominance and lateral bud induction is related to the balance between the concentrations of auxins, cytokinins, and gibberellins (Woolley and Wareing 1972). Control over these lateral buds through apical dominance may have been held by ramets other than the experimental ramet (McIntyre 1969, 1971, Phillips 1975, Jónsdóttir and Callaghan 1988). An alternative to the hypothesis of phyohormonal control is that lateral buds remained dormant due to a lack of resources and that rhizome severing released these resources to an extent that the experimental ramet was not able to retain control over these buds (McIntyre 1969, 1971).

It is generally accepted that sexual and clonal reproduction compete for limited resources and that allocation of resources to one form of reproduction will come at the expense of allocation to the other form (Abrahamson 1975, Williams 1975). Mayflower reproduces sexually through the production of seeds and reproduces vegetatively through the initiation of rhizomes. Each rhizome initiated may eventually become a new ramet. The hypothesis of reproductive competition would predict that ramets maturing more seeds would initiate fewer rhizomes than ramets maturing fewer seeds. Although not specifically tested, there does not appear to be much, if any, support for this hypothesis since there was no pattern of rhizome initiation among flowering ramets receiving an overabundance of pollen, natural levels of pollen, and no pollen, and for vegetative ramets which are not capable of producing seeds. This lack of pattern may be due to large differences in the stored resources among individual ramets. Ramets with fewer resources may not initiate rhizomes regardless of the resource demands of maturing seeds, and ramets with more resources available may have initiated more rhizomes regardless of resources allocated to seed maturation. In ramets of Clintonia borealis, another herbaceous clone that inhabits similar areas to mayflower, the increased production of flowering ramets was correlated with increased initiation of rhizomes (Pitelka, Hansen, and Ashmun 1985). Moreover, mayflower genets are typically linear and bifurcations are not common. Therefore the initiation of new rhizomes may be, to some extent, developmentally constrained.

CHAPTER III

DO RAMETS INCUR A REPRODUCTIVE COST OF PREVIOUS FLOWERING: A NATURAL EXPERIMENT

Abstract

A field experiment tested whether the number of seeds matured by Canada mayflower (Maianthemum canadense var. canadense Desf.) ramets was pollen limited and whether ramets flowering for the first time differed from ramets flowering for the second time in the number of seeds that they were able to mature. The addition of pollen resulted in an increase in the number of seeds matured by ramets and therefore the number of seeds matured by ramets was pollen limited. No difference in the number of seeds matured by ramets flowering for the first time and ramets flowering for the second time was detected and therefore previous flowering was not thought to affect the number of seeds matured by ramets in the future. There was, however, a negative relationship between the number of seeds that individual ramets matured and the mean weight of the ramet's seeds, indicating that ramets were experiencing resource limitation to some degree.

Introduction

Plants must allocate limited resources to structures associated with growth, defense, and reproduction (Bazzaz et al. 1987). Over a plant's lifetime, allocation patterns must balance fecundity and survivorship (Bazzaz et al. 1987). Therefore, allocation of resouces to reproduction in the current year may not only reduce growth in the current year, but may also reduce future fecundity and survivorship due to decreased size (Stephenson 1981, Lovett-Doust 1989).

The immutability of the "tradeoff" between current and future fecundity may be mitigated in some species (Snow and Whigham 1989): flowers and fruits may carry on their own photosynthesis (Bazzaz et al. 1979, Williams et al. 1985), plant structures may serve functions in addition to reproduction (Reekie and Bazzaz 1987), photosynthetic rates in nearby leaves may increase with reproduction (Reekie and Bazzaz 1987), and finally plants may be able to recover nutrients from reproductive structures (Whigham 1984, Chapin 1980). Despite the potential influence of these mechanisms, a tradeoff between current fecundity and subsequent growth and fecundity has been reported for the orchids Cypripedium acaule (Primack and Hall 1990), Epidendrum ciliare (Ackerman and Montalvo 1990), and Tipularia discolor (Snow and Whigham 1989). Current fecundity also reduced growth in mayapple (Podophyllum peltatum, Sohn and Policansky 1977; although see Benner and Watson 1989) and reduced the probability of becoming female in jack-in-the-pulpit (Arisaema triphyllum; Bierzychudek 1984).

As well as a plant's reproductive history, current fecundity in self-incompatible, insect-pollinated plants may be influenced by the availability of compatible pollen (Bierzychudek 1981, Thompson and Stewart 1981). This is not to suggest that a plant shown to be pollen limited in a given year is not ultimately resource limited (Bierzychudek 1981) as the addition of pollen in one year may increase fecundity at the expense of future fecundity (Janzen et al. 1980).

For some plants, such as many trees, size tends to increase over time as these plants accumulate biomass. For others, including many herbaceous clonal plants, the size of ramets (the functional units capable of reproduction) may be more or less fixed and the accumulation of biomass may result in the increase in size of the genet as additional ramets are produced. The consequence of this is that ramet size may not predict accurately reproductive success (Ashmun and Pitelka 1984). It may also be that genet size (the sum of all ramets of a particular genet) does not accurately predict reproductive success either. Connections between ramets may "break down" resulting in smaller subunits that themselves may have lower immediate reproductive success due to decreased size, although this may not mean lower lifetime reproductive success if the breakup of the genet increases ramet survivorships (Cook 1979, 1985, Eriksson and Jerling 1990).

The probability of detecting a relationship between current and future ramet fecundity in clonal plants is likely to be influenced by the degree of integration among ramets (Lovett-Doust 1989). Ramets that are essentially independent may experience their own "cost of reproduction" while an integrated ramet may have the cost mitigated by other ramets.

Canada mayflower (Maianthemum canadense var. canadense Desf.; Gleason and Cronquist 1991) is a rhizomatous, perennial herb with genets that consist of dimorphic ramets. Flowering ramets have 2–3 leaves with a terminal inflorescence consisting of 4–35 perfect flowers, while vegetative ramets have only 1 leaf. Mayflower is self-incompatible (Worthen and Stiles 1986; TD Lee unpublished data) and has been shown to be pollen limited in New Jersey (Worthen and Stiles 1988) and in southeastern New Hampshire (Chapter I).

In the fall, ramets "die back," leaving an overwintering bud that will produce the aboveground ramet in the following year. With the die back of the ramets, an abscission scar is left. The scar is distinctive for vegetative and flowering ramets, and it is possible to determine the age of the ramet and whether the ramet has been flowering or vegetative for each of its previous years (Silva et al. 1982). It is also possible to identify ramets that have flowered previously by the dead flowering stalk that is sometimes present (Ganger, unpublished data).

In a manipulative, field experiment, ramets that were open pollinated were able to mature an equal number of seeds whether their rhizomes were severed or left intact. However, ramets that were overpollinated matured more seeds when their rhizomes were left intact than when they were severed (Chapter I). In that experiment, mayflower ramets flowered for the first time when they were on average 3.97 years old (SD=2.03, n=194). Of the 47 found to have flowered more than once, the mean number of years separating these flowerings was 2.71 years (SD=1.50). No ramets were found to have flowered in two consecutive years (Chapter I). One hypothesis for the variability in the number of years separating flowerings is that greater success of sexual reproduction in one year necessitates a greater "recovery" time for the ramets. Alternatively, ramets that wait longer to flower may be able to acquire more resources and thus mature more seeds.

While individual mayflower ramets have been shown to be pollen limited overall, and resource limited if their rhizomes were severed and hand pollinated, it is not known whether ramets themselves bear a cost of having flowered in the past. If there is a cost, ramets flowering for the second time may mature fewer seeds than ramets flowering for the second time. This cost may also be reflected in the resources allocated to these seeds. Specifically, some ramets may allocate more resources to their seeds than other ramets. It is important to note that the ramets flowering for the second time are a subset of ramets that flowered previously. It may be that only ramets of higher "vigor" are capable of flowering more than once and as such these ramets may be predicted to mature a greater number of seeds than ramets flowering for the first time.

A field experiment was undertaken to address the following questions: Is mayflower pollen limited in local populations? Is there evidence of a cost of flowering such that ramets flowering for the first time are able to mature more seeds than ramets flowering for the second time? Are ramets that remain vegetative for a longer period of time able to mature more seeds? Is there evidence of resource limitation such that the number of seeds a ramet matures is related to the mean weight of these seeds, and is this relationship different for ramets flowering for the first and second time?

Methods

In the summer of 1996, 60 ramets that were flowering for the first time were identified as well as 60 ramets that were flowering for the second time. These two "flowering" treatments represented one of the factors in a two-factor experiment. Half of the flowering ramets in each of these treatments were hand pollinated while the other half of the flowering ramets were open pollinated. These two treatments formed the "pollination" factor. The experiment was conducted in a mixed coniferous-hardwood canopy forest within the University of New Hampshire woodlands, Durham, NH.

Each of the flowers of the hand-pollinated ramets were pollinated each day for the life of the flowers. Pollen was collected immediately prior to use from 10–20 flowering ramets not more than 20 m away and applied to the flowers with a wooden toothpick. All of the flowers in one of the first-time flowering treatments and in one of the second-time flowering treatments were open pollinated and presumably received natural levels of pollination. At the end of the fruiting season, all of the flowering ramets were excavated and taken to the laboratory. The numbers of fruits and seeds matured by each ramet were noted. Seeds were dried for 72 hours at 80°C in a drying oven and then weighed to the nearest 10^{-5} g using a Mettler AE 163 balance. The mean weight of seeds per ramet was calculated. For each of the ramets that flowered for the first time, the age and number of years prior to flowering was noted. For each of the ramets that flowered for the second time, the age and number of years since flowering was noted.

A two-way analysis of variance (ANOVA) was used to test whether ramets flowering for the first time matured more seeds than ramets flowering for the second time and whether ramets were pollen limited. The statistical model consisted of two factors: flowering (first time flowering vs. second time floweing) and pollination (overpollinated vs. open-pollinated). Both factors were categorical and the dependent variable was the number of seeds matured. If there was an effect of previous flowering on the number of seeds matured, then this effect was more likely to be observed in ramets that received an overabundance of pollen. Therefore, one a priori contrast was considered: the number of seeds matured by overpollinated, first-time flowering ramets was compared to the number of seeds matured by overpollinated, second-time flowering ramets.

In order to determine if ramets that remained vegetative for a longer period of time were able to mature more seeds, two regressions were performed. For overpollinated, first-time flowering ramets, the number of seeds that they matured was regressed on the number of years prior to flowering. For overpollinated, second-time flowering ramets, the number of seeds they matured was regressed on the number of years since flowering.

The final experimental question involved the relationship between the number of seeds a ramet matured and the mean weight of these seeds, and whether this relationship varied between first- and second-time flowering ramets. If all of the flowers of an inflorescence were pollinated simultaneously and fruits developed synchronously, then the mean weight of the seeds per ramet might be dependent on the number of seeds. Since the flowers of a mayflower inflorescence open sequentially, the number of seeds may be dependent on the mean weight of these seeds. For example, if one of the early-developing seeds were able to garner a large amount of resources then the development of seeds by later-pollinated flowers may be prevented (Lee 1988). In order to determine whether there was a relationship between the number of seeds matured by a ramet and the mean weight of these seeds and whether this relationship varied between first- and second-time flowering ramets, an analysis of covariance (ANCOVA) was performed. The number of seeds was the dependent variable. The number of times flowering was the independent, categorical variable and the mean weight of seeds per ramet was the covariate. In this analysis, only those ramets that matured seeds were considered.

Results

Forty-six of sixty open-pollinated ramets matured one or more seeds while 51 of 60 overpollinated ramets matured one or more seeds. Six ramets were excluded from analyses because they were found to have flowered more than twice or they could not be accurately aged or their reproductive history determined due to decay.

Ramets that were overpollinated matured more seeds than ramets that were open pollinated ($F_{1,107,0.05}=11.157$, $p<0.005$, $r^2=0.112$; Fig. 12). There was no difference in the

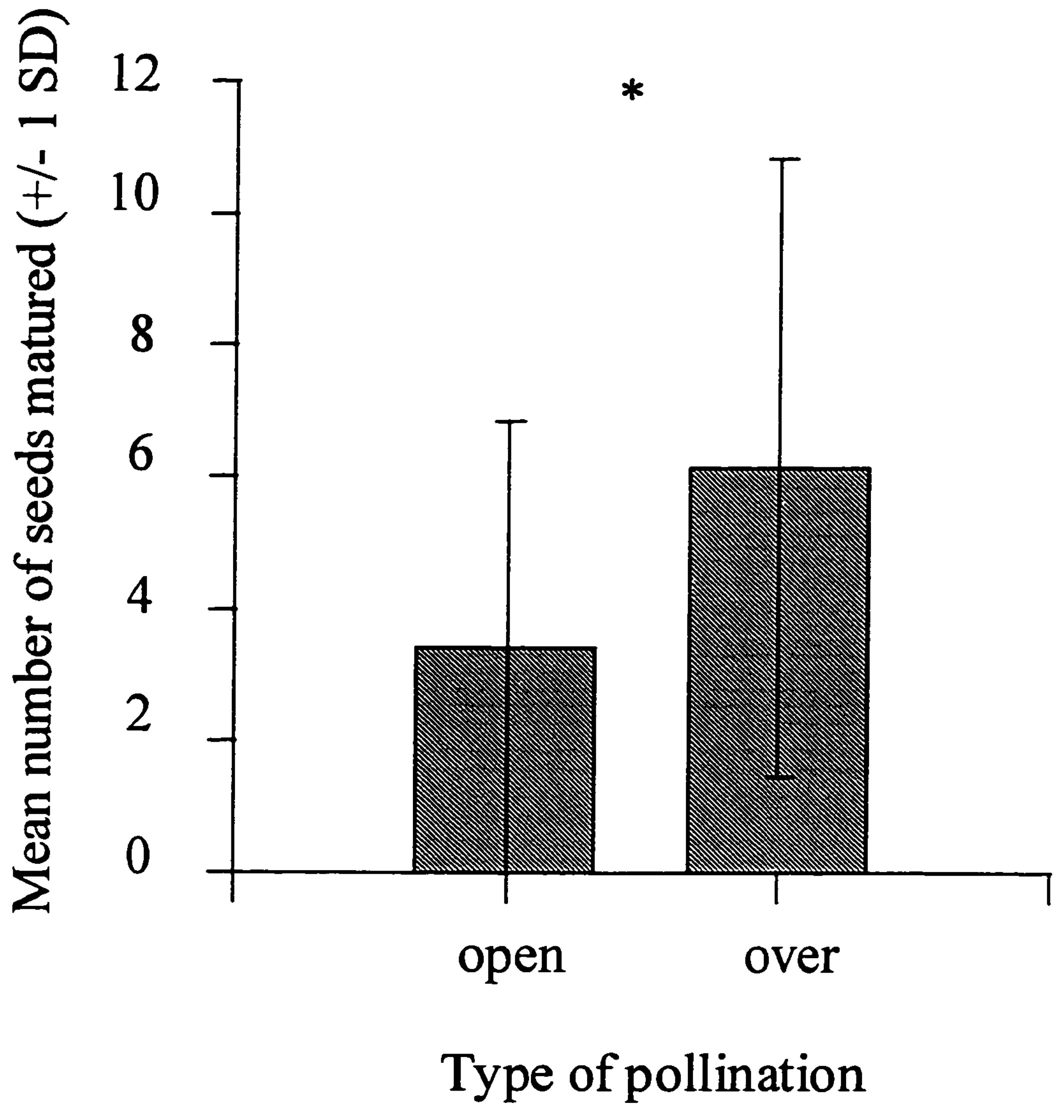

Figure 12. The mean number and standard deviation (SD) of seeds matured by open-pollinated ramets (open) and overpollinated ramets (over).

number of seeds matured by ramets flowering for the first and second time ($F_{1,107,0.05}$=0.503, p=0.480; Fig. 13). There was also no interaction between the pollination and flowering treatments ($F_{1,107.0.05}$=0.898, p=0.345). The a priori contrast was also not significant; there was no difference between the number of seeds matured by overpollinated, first-time flowering ramets and overpollinated, second-time flowering ramets ($F_{1,106,0.05}$=0.630, p=0.429). In concluding that there was no difference between these two treatments, there is an associated probability of being wrong. This is the Type II error or ß. Following Winer et al. (1991) it was possible to determine ß and therefore the statistical power of this comparison (1-ß) given specific alternative hypotheses. The statistical power of the comparison was determined assuming a true difference between the treatments of 1, 2, and 3 seeds (Table 4). Statistical power for the alternative hypothesis of 1 seed difference between the treatment means was low (power=0.208) and only with an alternative hypothesis of 3 seeds difference did the statistical power become greater than 0.80.

The average number of years prior to flowering for ramets flowering for the first time, was 3.94 years (SD=1.81) and the average age of these ramets was 4.94 years (SD=1.81). For ramets flowering for the second time, the average number of years since first flowering, was 2.88 years (SD=0.75) and the average age of these ramets was 8.41 years (SD=1.87). The number of seeds matured by overpollinated ramets was not correlated with the number of years prior to flowering for first-time flowering ramets ($F_{1,20,0.05}$=0.533, p=0.47) or with the number of years since flowering for second-time flowering ramets ($F_{1,28,0.05}$=2.336, p=0.14).

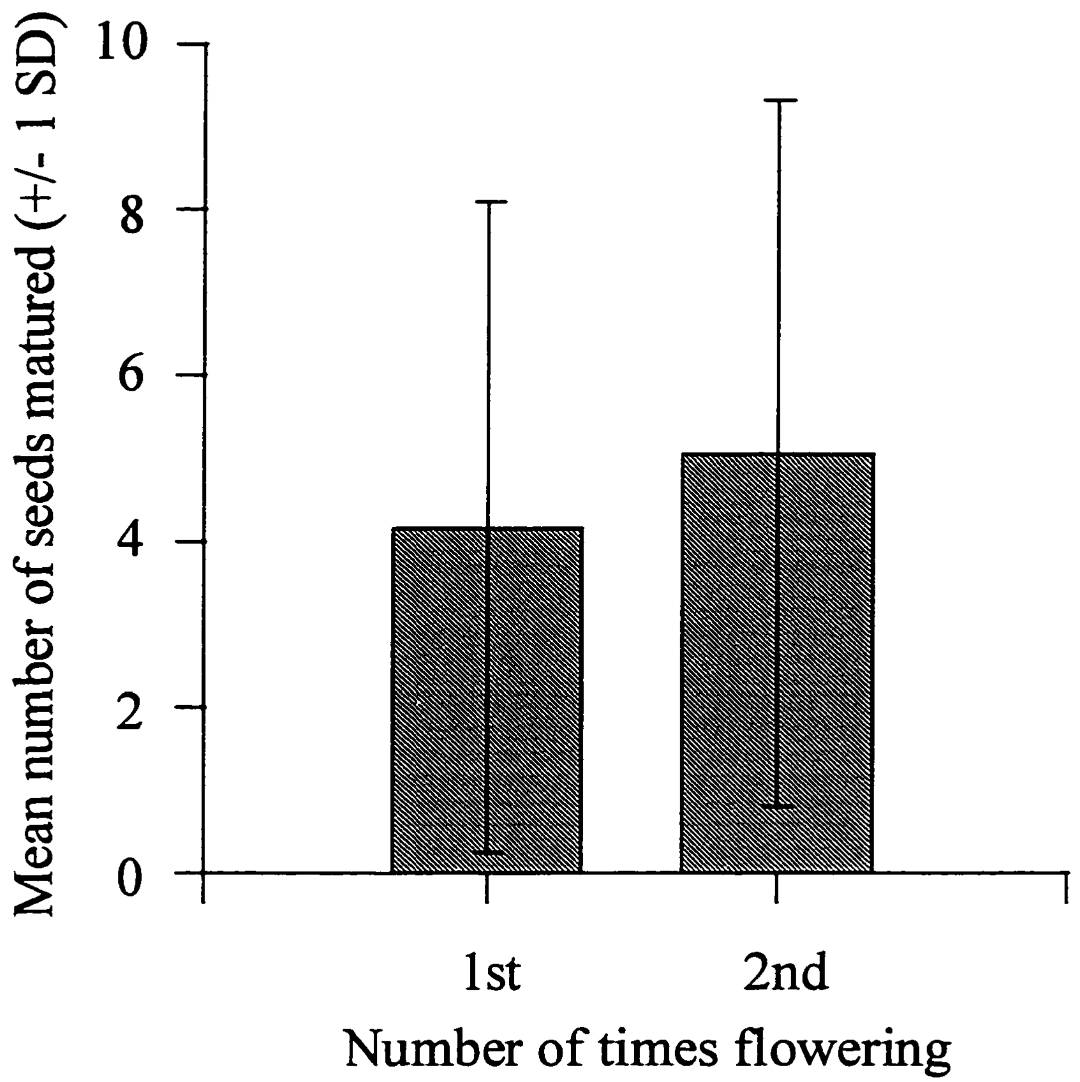

Figure 13. The mean number and standard deviation (SD) of seeds matured by overpollinated ramets flowering for the first time (1st) and overpollinated ramets flowering for the second time (2nd). This is the a priori contrast.

Table 4. The probability of making a Type II error (ß) and the statistical power for three alternative hypotheses comparing the number of seeds matured by overpollinated, first-time flowering ramets and overpollinated, second-time flowering ramets.

Alternative hypothesis: $\mu_1-\mu_2=$	ß	power (1-ß)
1 seed	0.792	0.208
2 seeds	0.491	0.509
3 seeds	0.197	0.803

Seeds varied in weight from 0.00328g to 0.0180g. Overall, there was a negative relationship between the number of seeds matured by ramets and the mean weight of these seeds ($F_{1,40,0.05}$=9.855, p<0.005, r^2=0.217). There was no difference in this relationship for ramets flowering for the first (Fig. 14) or second time (Fig. 15) either in the slopes of this relationship ($F_{1,40,0.05}$=0.105, p=0.747) or in the y-intercepts ($F_{1,41,0.05}$=1.428, p=0.239).

Discussion

The addition of pollen resulted in an increase of, on average, 2.7 seeds. This suggests that ramets were pollen limited during the experiment and this represents the third consecutive year that mayflower ramets were experimentally demonstrated to be pollen limited at this site (Chapter I).

Ramets matured comparable numbers of seeds regardless of whether this was their first- or second-time flowering. Thus the act of flowering did not appear to influence the number of seeds matured in the future. This was true as well for ramets that received an overabundance of pollen. Statistical power was low for a hypothesized difference of 1 seed. However at a hypothesized difference of 3 seeds (approximately the difference between the pollination treatment means) power was quite high. While it was possible to determine if ramets had previously flowered, it is important to note that nothing is known about the success of previous flowering. It could be that the number of seeds matured in the past was low and therefore the costs incurred by ramets at that time were also low. The cost of having reproduced before may also have been mitigated by other ramets at the time of flowering. Mayflower ramets are known to mature a greater number of seeds if

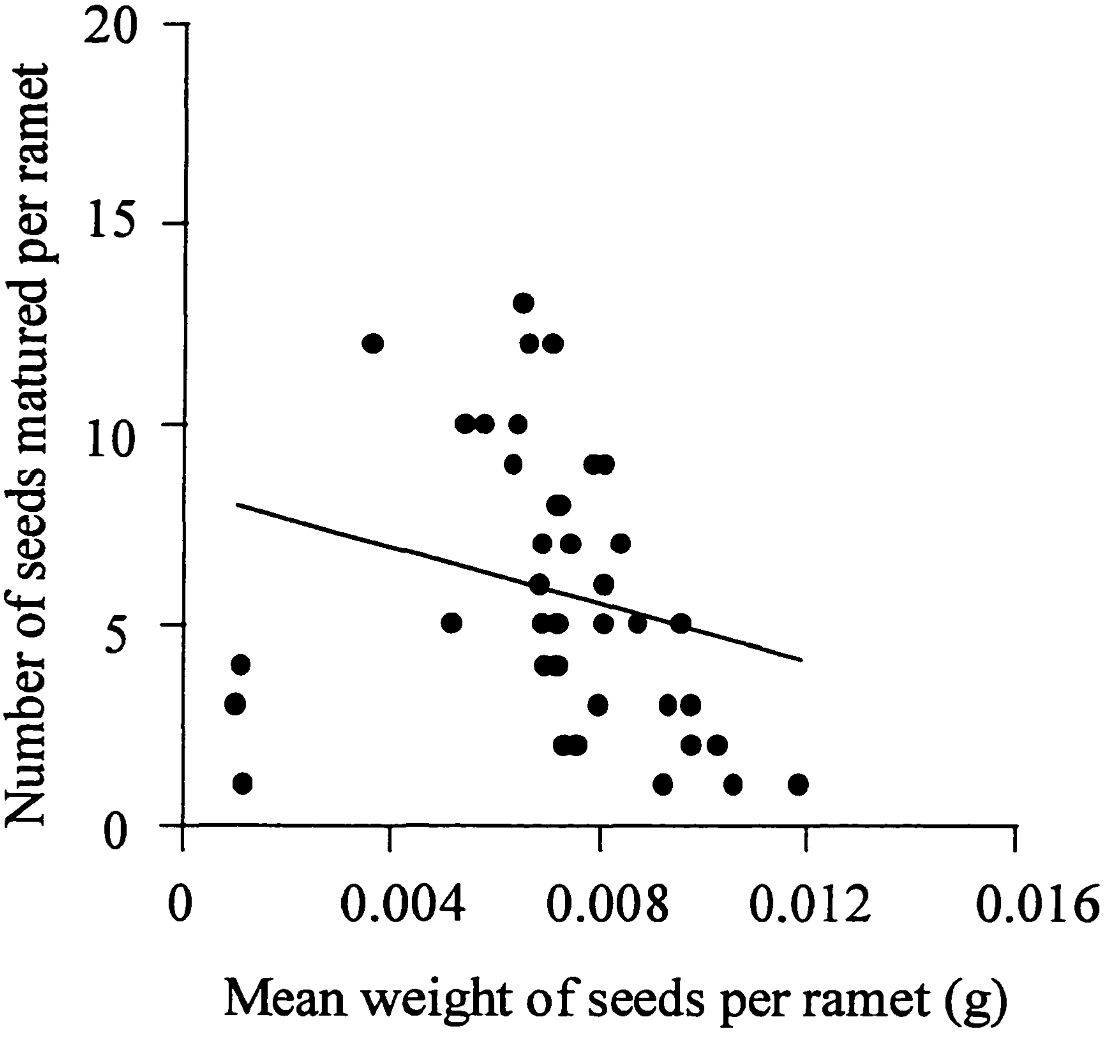

Figure 14. The relationship between the number of seeds matured per ramet and the mean weight of these seeds for ramets flowering for the first time.

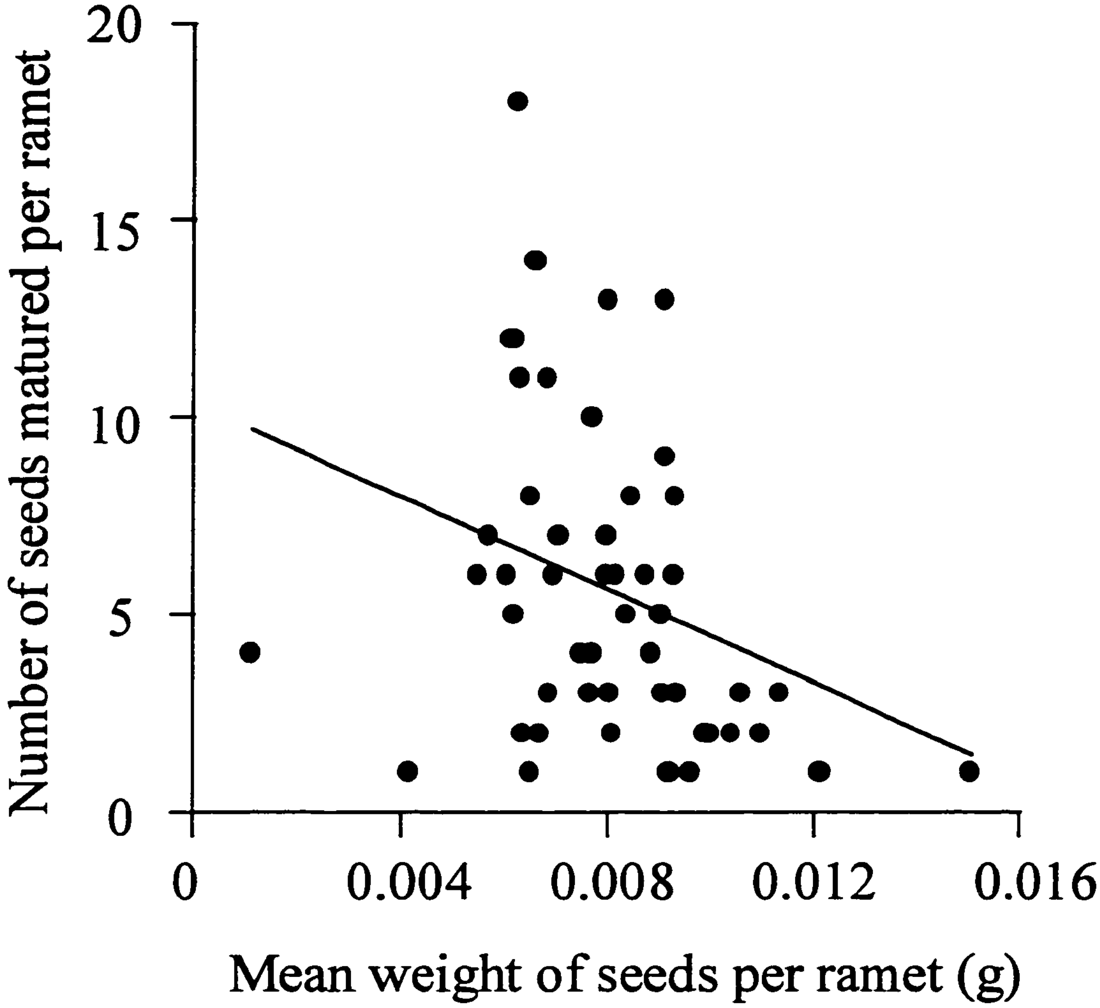

Figure 15. The relationship between the number of seeds matured per ramet and the mean weight of these seeds for ramets flowering for the second time.

they receive ample pollen and if their rhizomes are left intact (Chapter I). This suggests that resources are translocated to the flowering ramet, either from the rhizome or from other ramets, and may offset the cost of reproduction incurred by the ramet. There are alternative ways in which the cost of reproducing may be expressed other than an effect on future sexual reproduction. Ramets may experience reduced rhizome growth, reduced storage of resources, and reduced survivorship.

While the lack of an effect of previous flowering on the number of seeds matured currently was not evident, it appears that overall the ramets did experience resource limitation—even when ramets that were pollen limited were included. The number of seeds that ramets matured was negatively related to the mean weight of these seeds indicating that ramets were allocating limited resources. The cost of maturing heavier seeds was a decrease in the number of seeds matured. That this relationship was similar for ramets flowering for the first and second time further suggests that there was not a cost of having flowered in the past.

Mayflower is not the only example of plants in which a cost of previous flowering was not detected and in fact some plants show an increased likelihood of future flowering with greater current reproductive allocation. In the orchid *Spiranthes cernua*, Antlfinger and Wendel (1997) found that there was an overall trend of individuals producing fewer flowers being less likely to flower in the following year than individuals producing more flowers. This was despite the fact that individuals producing many flowers had a tendency to decrease in size in the following year. In the early spider orchid *Ophrys sphegodes*, Hutchings (1987) found that individuals flowering in the current year were: 1) more likely to flower in the following year than either vegetative or dormant individuals, 2) less likely to enter dormancy than either vegetative or dormant individuals, and 3) had a smaller chance of dying than dormant individuals.

Other plants show no effect of current flowering on future reproduction. Horvitz and Schemske (1988) experimentally created high and low levels of reproductive effort in Calathea ovandensis and found no difference in the growth, survival, and reproduction of individuals between treatments in the following year. Smith and Yound (1982) found that individuals of Senecio keniodendron that had high levels of reproduciton were more likely to die than other individuals. However, those that did survive were found to have higher levels of reproduction than other individuals in the following year.

For mayflower, the number of years prior to flowering for first-time flowering ramets and the number of years since first flowering for second-time flowering ramets were not correlated with the number of seeds matured. The number of years since flowering may represent the combination of two variables: 1) the cost incurred by the ramet for past flowering and the number of seeds matured (this may be influenced by the degree of ramet integration) and 2) the quality of the habitat. In other words, a ramet that waited a greater number of years to flower again may have incurred a great cost of previous reproduction, one that the genet was not able to offset, or the quality of the habitat was such that resources were able to be replenished only very slowly or even some combination of the two. It may also be that another part of the genet was flowering during the years between flowerings and that the ramet in question was subsidizing the number of seeds matured in another part of the genet.

CHAPTER IV

RAMET CONTEXT AND RAMET-SPECIFIC VARIABLES: A COMPARISON OF THEIR EFFECT ON THE NUMBER OF SEEDS MATURED BY A CLONAL HERB

Abstract

A study was undertaken with Canada mayflower (Maianthemum canadense var. canadense Desf.) to determine if the number of seeds matured by ramets was related to ramet-specific variables and ramet context. The ramet-specific variables investigated were the density of flowering ramets, the distance to the nearest-neighbor flowering ramet, the number of times that the ramet flowered, and the number of years prior to/since flowering. Context was defined as the identities of the ramets directly acropetal and basipetal on the same rhizome system. The number of seeds matured by ramets was not related to any of the ramet-specific variables. The number of seeds matured by ramets, however, was found to be related to context.

Introduction

Clonal plants (genets) grow through the iterative production of ramets. These ramets may be of various ages and reproductive histories and may be situated in locations with different edaphic and climatic conditions (Marshall 1990). Despite this spatial separation, ramets may be physiologically integrated and resources may be translocated between them (Pitelka and Ashmun 1985). Young ramets are thought to develop with subsidization from older ramets, pass into a period of relative independence, and eventually subsidize younger ramets (Pitelka and Ashmun 1985, Marshall 1990, Price and Hutchings 1992). For many plants, this developmental program may be altered during periods of "stress" or high resource demand (Marshall 1990). For example, younger ramets may subsidize stressed older ramets. The ability of ramets to subsidize one another is constrained by the plant's phyllotaxy (Bell and Tomlinson 1980; Price, Marshall, and Hutchings 1992) as well as the distance between ramets (Marshall 1990). In order for resources to be translocated, the xylem and phloem must connect the ramets and the distance between them should not be such that the cost of translocation exceeds the value of the resources themselves (Marshall 1990).

Determining which ramets in a population are part of which genets is difficult. Genets are often extensive and diffuse (Cook 1985). Connections between ramets may decay over time and even those ramets that remain physically connected may not always be physiologically integrated. Due to this, researchers have attempted to describe the demography of clonal plant populations through the demography of ramets (Cook 1985). Theoretically, conclusions based on ramet demography can oppose conclusions based on genet demography for the same population (Cook 1985).

If the survivorship and reproduction of a ramet is influenced by other ramets within the same genet, then adjacent ramets may be more greatly affected than are more distant ramets. This would especially be true of a ramet on a monopodial rhizome system whose phyllotaxy connects it to these proximate ramets. The identity of these proximate ramets as well as their age and reproductive history is termed the “context” of a focal ramet. On a monopodial rhizome system, the context of a focal ramet could be described by the identity of the ramet directly acropetal and the ramet directly basipetal along that rhizome system (Fig. 16).

There may be other variables important in determining the survivorship and reproduction of a given ramet that are not related to the ramet's context. These may include the ramet's own reproductive history, its age, the local density of ramets, and local microenvironment variables. To the extent that these other variables are important, the use of a ramet-based approach may be adequate in determining the demography of a population of clonal plants. To the extent that the ramet's context is important, the use of a genet-based approach may be required to make accurate predictions concerning the clonal plant population.

Canada mayflower (Maianthemum canadense var. canadense Desf.; Gleason and Cronquist 1991) is a rhizomatous, perennial herb common to the understory of boreal forests. The ramets of mayflower are dimorphic and may be either vegetative (1 leaf) or flowering (2–3 leaves with a terminal inflorescence consisting of 4–35 perfect flowers). Mayflower is self-incompatible (Worthen and Stiles 1986) and local populations of mayflower have been demonstrated to be pollen limited (Chapter I). The ramets of mayflower are potentially long-lived and it is possible to determine the age of the ramet and the type of ramet (vegetative or flowering) that it was in each of its previous years (reproductive history) (Silva et al. 1982). Given this record, it is possible to determine the number of times that a flowering ramet has flowered during its lifetime and the number of years prior to flowering for ramets that are currently flowering for the first time or the number of years since flowering for ramets that are currently flowering for the nth time.

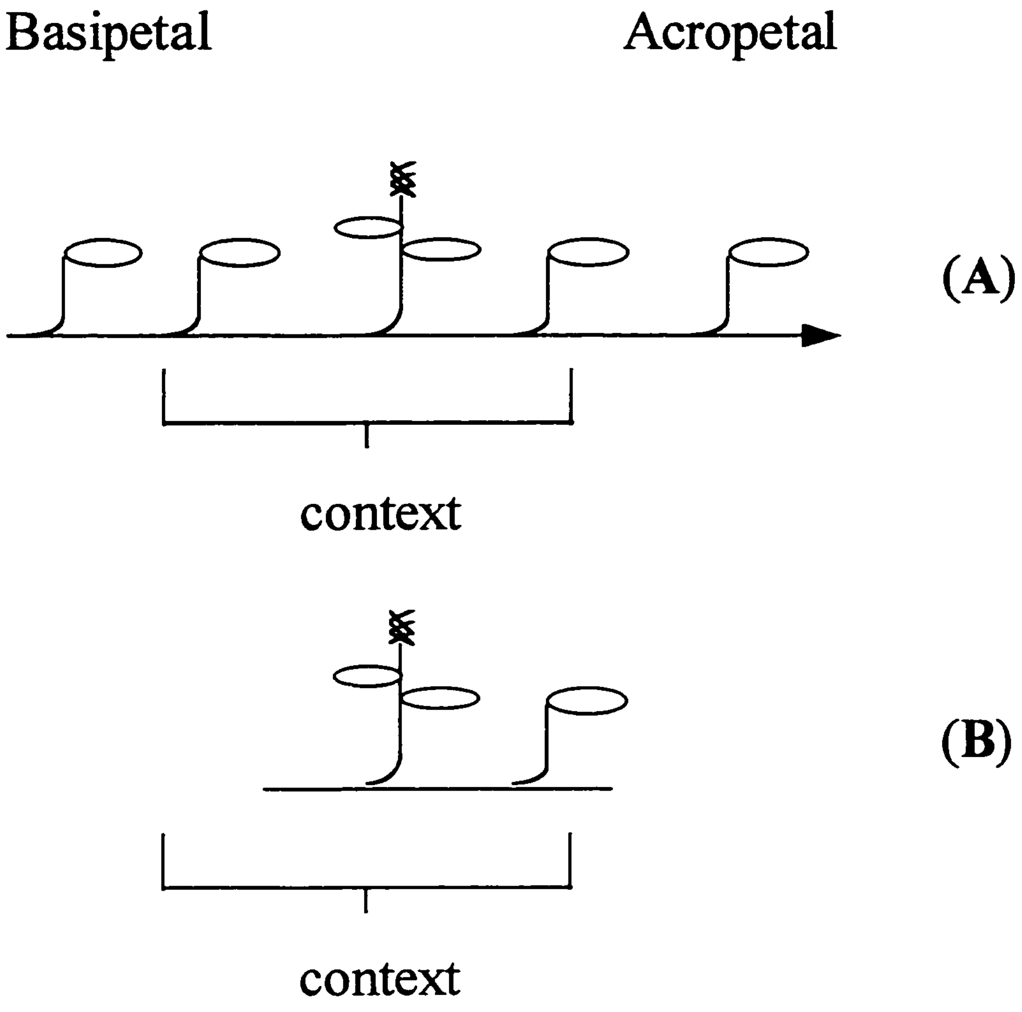

Figure 16. Two contexts are presented for two flowering ramets. A This flowering ramet has a vegetative ramet both basipetal and acropetal while B this flowering ramets has no ramet basipetal and a vegetative ramet acropetal.

A study was conducted to determine if ramet context and ramet-specific variables were related to the number of seeds matured and flowering bud production in mayflower. The following questions were addressed in the study: Is the surface area of vegetative leaves related to the age of the ramet and/or the production of a flowering bud? Is the success of a ramet's current sexual reproduction related to local factors, to the ramet's reproductive history, and/or to the ramet's context? Does the ramet's context correlate with ramet age and are flowering ramets older than vegetative ramets?

Methods

Work was conducted in the University of New Hampshire woodlands in Durham, NH where mayflower common throughout the mixed deciduous-coniferous forest. Ramets tend to occur in patches and it is possible that a patch of mayflower would contain plants in a similar stage of development. Therefore ramets were collected from 53 locations within a three hectare area of the woodlands. Locations were separated by at least 20 m. Two ramets, a flowering ramet and a vegetative ramet, were selected that were closest to a randomly-placed stake within each location. The stake's location was determined by throwing it along a random compass bearing.

Information concerning local factors was collected at each location. The density of flowering ramets within a 1m^2 circle centered on the chosen flowering ramets was determined. The density of both vegetative and flowering ramets within a 0.25m^2 circle centered on the chosen flowering ramet was also determined. The distance from the chosen flowering ramet and the nearest flowering ramet was measured.

Both the chosen vegetative and flowering ramets were excavated to include any ramets acropetal and basipetal along the same rhizome. Bifurcations in the rhizome were excavated as well. In the laboratory, the age and reproductive history of all ramets was determined following Silva et al (1982). The number of fruits and seeds matured by each flowering ramet was also determined. The leaf surface area of each vegetative ramet was estimated. Estimations were based on an established relationship between the leaf surface area and two linear measures of the leaf. The length (L) from the tip of the leaf to the base of the leaf and the width (W) of the leaf at the midpoint of the length together accurately predict leaf surface area (area = 27.778*L + 54.998*W - 1198.16; $p<0.001$, $r^2=0.936$; Ganger, unpublished data)

An analysis of variance (ANOVA) was used to determine if bud type and the estimated leaf surface were related. It is not known if the bud type produced is dependent on the leaf surface area or vice versa. Therefore the estimated leaf surface area was chosen as the dependent variable and the bud type as the independent, categorical variable. In order to determine if leaf surface area increased with age, the estimated leaf surface area was regressed on age.

In order to determine if the number of seeds matured was related to local factors, a multiple regression was performed: the number of seeds matured by each ramet was regressed on the local density of flowering ramets, the local density of all ramets, and the nearest flowering neighbor. In order to determine if the success of sexual reproduction was related to the ramet's reproductive history, the number of seeds matured by each ramet was regressed on the number of times that the ramet flowered during its lifetime and the number of years prior to the flowering in the case of first time flowerers or the number of years since the last flowering occurrence in the case of ramets that flowered more than once during their lifetime.

Vegetative and flowering ramets were classified according to the following schedule: vegetative ramet basipetal, flowering ramet basipetal, no ramet basipetal (decayed rhizome), or dead ramet basipetal. This classification was termed the basipetal context. The same ramets were assigned to classes according to the following schedule: vegetative ramet acropetal, flowering ramet acropetal, no ramet acropetal (decayed rhizome), dead ramet acropetal, or growing rhizome. This classification was termed the acropetal context. The flowering ramets were also assigned according to the following schedule: 1) dead ramet or no ramet basipetal and dead ramet or no ramet acropetal, 2) vegetative ramet basipetal and dead ramet or no ramet acropetal, 3) dead ramet or no ramet basipetal and vegetative ramet acropetal, 4) vegetative ramet basipetal and vegetative ramet acropetal, or 5) flowering ramet either basipetal or acropetal. This classification, which contained information on both the acropetal and basipetal ramets, was termed context. If any class contained fewer than two observations, then it was dropped from the analysis. In order to determine whether the success of current sexual reproduction was related to ramet context, an ANOVA was performed with the number of matured seeds as the dependent variable and the context as the independent, categorical variable. In order to determine whether ramet age was related to ramet context and if vegetative ramets and flowering ramets differed in ages, an ANOVA was performed with the ages of both the vegetative and flowering ramets as the dependent variable and the ramet type (vegetative vs. flowering), the basipetal context, and the acropetal context as the three independent, categorical variables.

Each question posed concerning mayflower required a different statistical analysis and it was not possible to perform all analyses simultaneously. This was due to the fact that there are three populations of interest: flowering ramets, vegetative ramets, and both types of ramets combined. There were also specific variables, such as estimated leaf surface area, considered in different analyses as dependent and independent variables. Because several analyses were used, the probability of making a Type I error was increased (Winer et al. 1991). The results therefore should be viewed with this in mind and future experiments should be conducted to validate any demonstrated relationships.

Results

The average number of flowering ramets withing the m^2 surrounding the selected ramet was 12.4 (SD=12.75 ramets, range=1-66 ramets), while the average number of flowering and vegetative ramets per $0.25m^2$ was 44.4 ramets (SD=22.45 ramets, range=5–99 ramets). The average distance from the chosen flowering ramet and the nearest flowering ramet was 28.2 cm (SD=22.41 cm, range=4.4–107 cm). Ramets on the same rhizome were separated on average by 23.5 cm (SD 7.28 cm, range=10.9–50.1 cm). Three vegetative and three flowering ramets could not be aged due to decay.

There was a relationship between the bud type and the estimated leaf surface area of vegetative ramets ($F_{1,50,0.05}=15.380$, $p<0.0005$, $r^2=0.254$; Fig. 17). Variances were homogeneous (F_{max} test, $p>0.05$; Winer et al. 1991). In addition to the 53 vegetative ramets collected, an additional 43 vegetative ramets were collected. These 43 vegetative ramets were the basipetal and acropetal vegetative ramets collected as part of the excavations. These 43 ramets were added to the original 50 and the analysis was

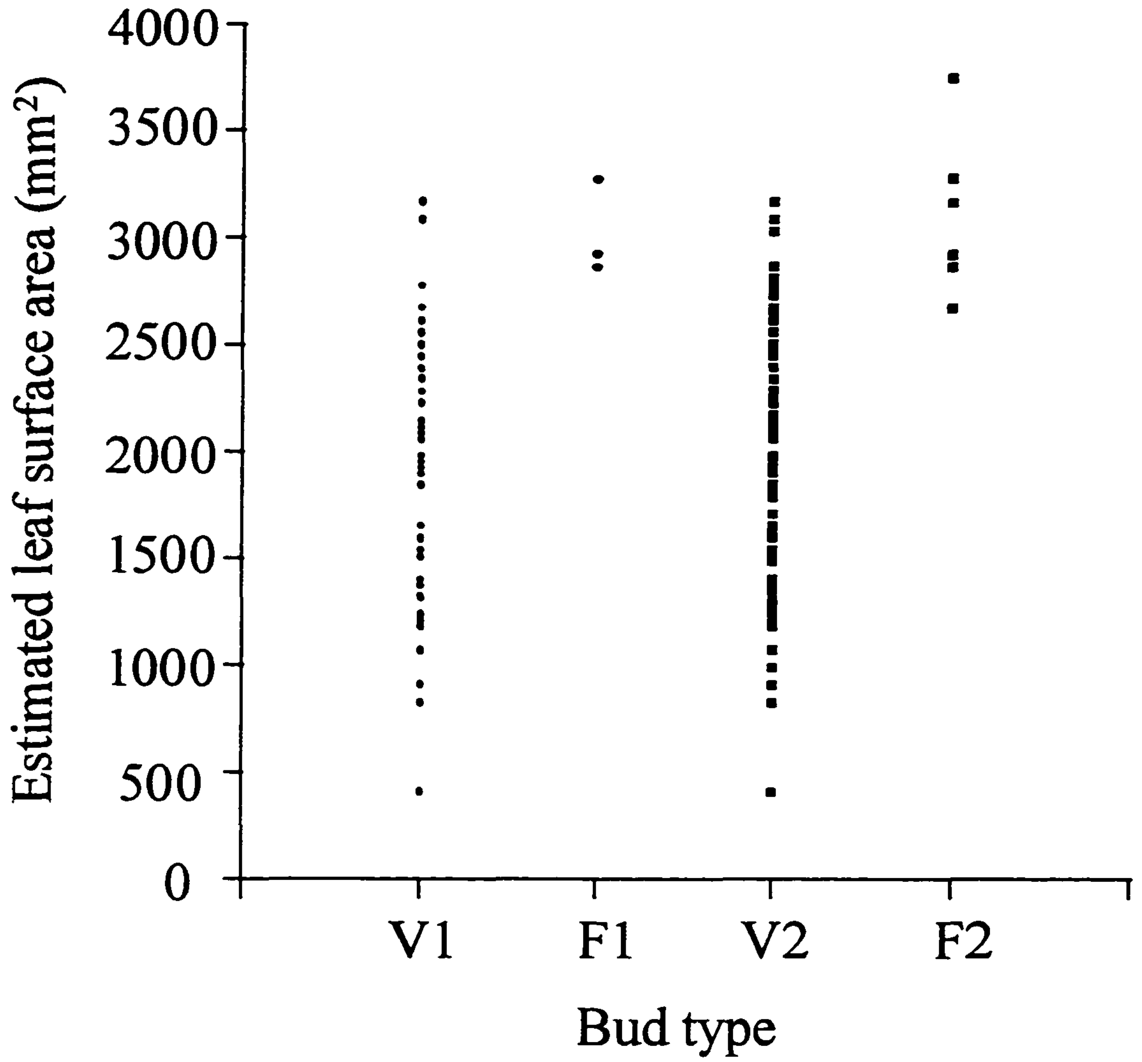

Figure 17. Plot of the bud types (vegetative, V, or flowering, F) by the ramet's estimated leaf surface area. The V1 and F1 represent only the 53 focal ramets. The V2 and F2 represent the 53 focal ramets plus any vegetative ramets basipetal or acropetal (43).

performed again. Note that it is possible that some genets may be overrepresented compared to other genets. With the additional observations, the bud type and the estimated leaf surface area were still related ($F_{1,94,0.05}$=36.207, $p<0.001$, r^2=0.288; Fig 17).

The estimated leaf surface area of vegetative ramets was not related to age ($F_{1,48,0.05}$=0.605, p=0.440). The addition of the other 43 vegetative ramets did not change the results ($F_{1,92,0.05}$=0.030 , p=0.864)

The number of seeds matured by flowering ramets was not related to the local density of flowering ramets (p=0.830), to the local density of all ramets (p=0.994), or the distance to the nearest flowering ramet (p=0.867). The number of seeds matured by flowering ramets was also not related to ramet reproductive history, including both the number of times that a ramet flowered ($F_{3,47,0.05}$=0.761, p=0.521) and the number of years prior to/since flowering ($F_{1,47,0.05}$=2.591, p=0.114). There was a significant difference between the number of years prior to flowering for ramets flowering for the first time (mean=3.8 years, SD=1.57 years, n=38) and the number of years since flowering for ramets flowering for the second time (mean=1.7 years, SD=0.500 years, n=9; $F_{1,45,0.05}$=16.139, $p<0.0005$, r^2=0.264).

Of the 53 flowering ramets collected, 26 of them had a dead ramet or no ramet basipetal and acropetal, 12 had a dead ramet or no ramet basipetal and a vegetative ramet acropetal, 3 had a vegetative ramet basipetal and a dead ramet or no ramet acropetal, 3 had a vegetative ramet basipetal and a vegetative ramet acropetal, and 5 had a flowering ramet either basipetal or acropetal. The number of seeds matured by flowering ramets differed with context ($F_{4,47,0.05}$=4.161, $p<0.01$, r^2=0.262). Specifically, flowering ramets that had a dead ramet or no ramet basipetal and a vegetative ramet acropetal matured more seeds than flowering ramets that had a dead ramet or no ramet basipetal and acropetal (Table 5; Fig. 18).

Of the 26 flowering ramets that had a dead ramet or no ramet basipetal and acropetal, 17 of these had no ramet basipetal or acropetal due to decayed rhizome. The number of seeds that these ramets matured was positively related to the total distance of rhizome basipetal and acropetal ($F_{1,16,0.05}$=20.480, $p<0.001$, r^2=0.549; Fig. 19).

Flowering and vegetative ramets were grouped according to their basipetal context and then separately according to their acropetal context. Of the 100 ramets collected, 12 of these ramets had a dead ramet basipetal, 18 of these ramets had a vegetative ramet basipetal, and 70 of these ramets had no ramet basipetal. Of the same 100 ramets collected, 10 of these ramets had a dead ramet acropetal, 37 had a vegetative ramet acropetal, 34 had no ramet acropetal, 5 had a flowering ramet acropetal, and 14 had a new rhizome/bud acropetal. Of the 14 ramets that produced a new rhizome/bud, 13 of these were from a vegetative ramet (mean age of ramet=2.39 years, SD=1.61 years) and only 1 of these was from a flowering ramet (age of ramet=12 years). Ramet ages differed significantly between ramet types ($F_{1,92,0.05}$=14.330, $p<0.0005$), among basipetal contexts ($F_{2,92,0.05}$=3.589, $p=0.0316$), and among acropetal contexts ($F_{4,92,0.05}$=3.121, $p=0.0187$). Flowering ramets averaged 6.2 years (SD=3.15 years) and were older than vegetative ramets which averaged 3.4 years (SD=2.14 years; Fig. 20). Ramets with no ramet basipetal were older than ramets with a vegetative ramet basipetal ($p=0.009$; Fig. 21A; Table 6). Ramets with a flowering ramet acropetal were older than ramets with a dead ramet acropetal ($p=0.032$), a vegetative ramet acropetal ($p=0.0109$), or ramets with a

Table 5. The mean number and standard deviation (SD) of seeds matured by flowering ramets in each of the contexts. The vegetative ramet basipetal and a dead ramet or no ramet acropetal class was not included since only one ramet in this class matured any seeds. Small case letters denote means which are similar. 0 = dead ramet or no ramet, v = vegetative ramet, f = flowering ramet.

Context	n	Mean	SD
0,0	21	2.95	2.31 a
0,v	12	5.50	3.75 b
v,v	3	5.67	3.51 a,b
f	3	3.00	2.00 a,b

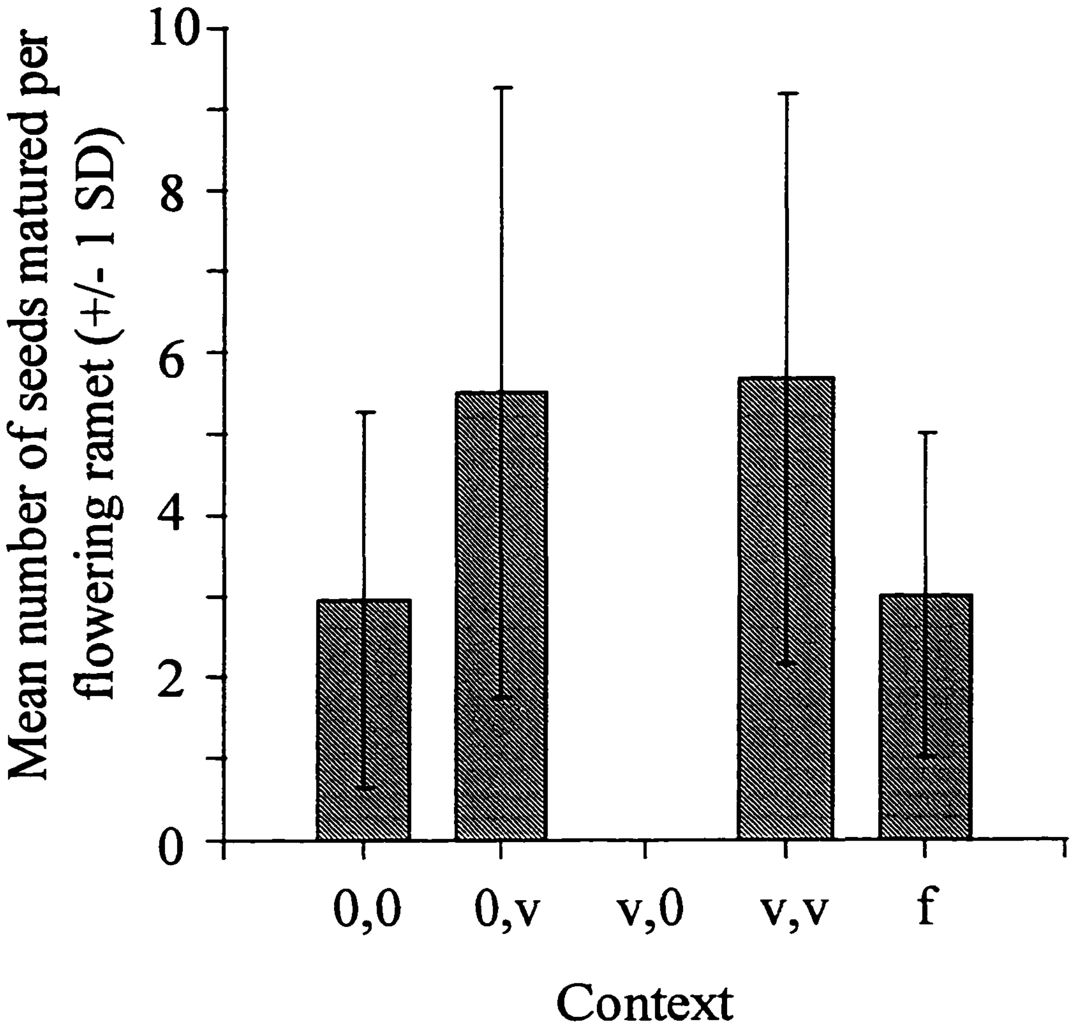

Figure 18. The mean number and standard deviation (SD) of seeds matured by mayflower ramets in each of the ramet contexts. 0 = dead ramet or no ramet, v = vegetative ramet, and f = flowering ramet.

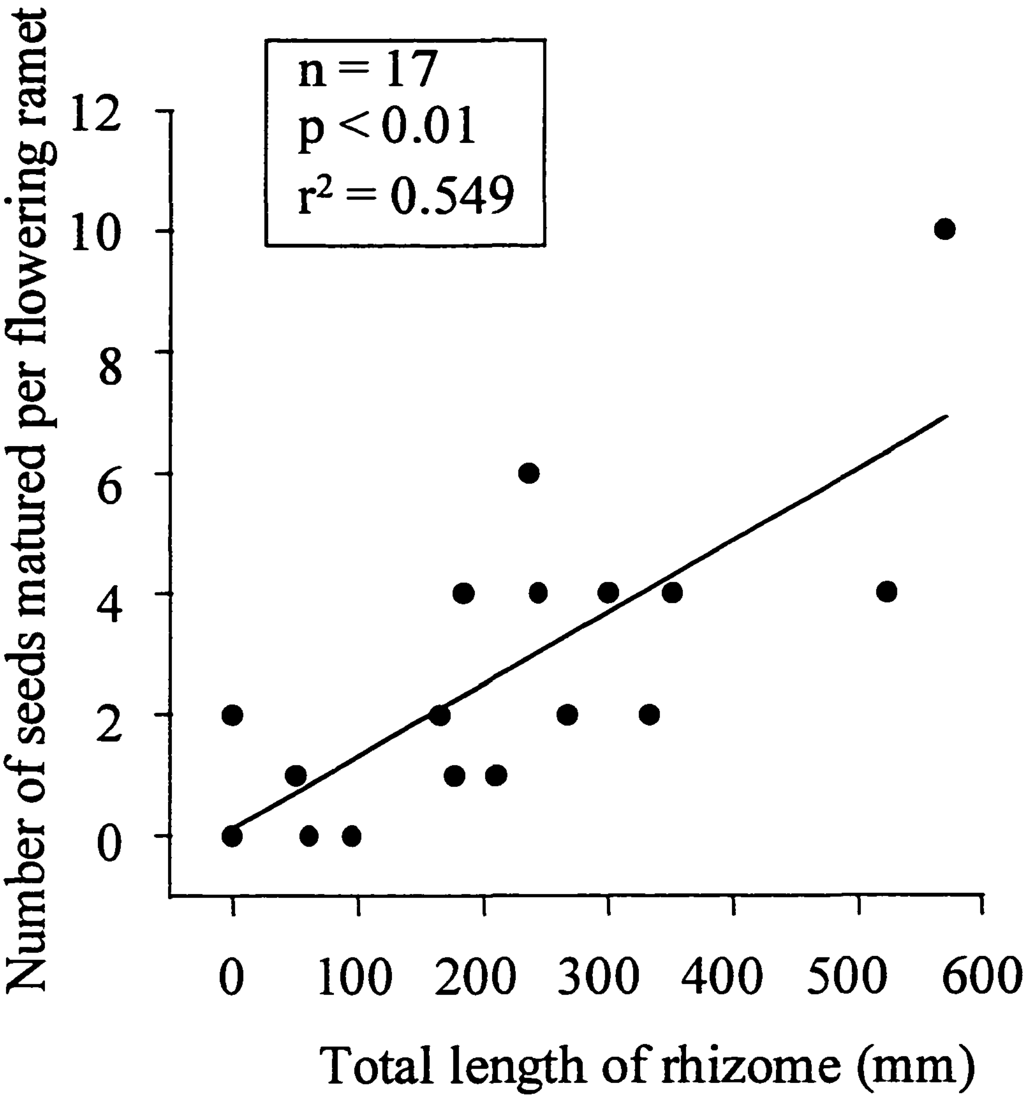

Figure 19. Plot of the number of seeds matured by the total length of rhizome available for ramets in the no ramet acropetal and basipetal context.

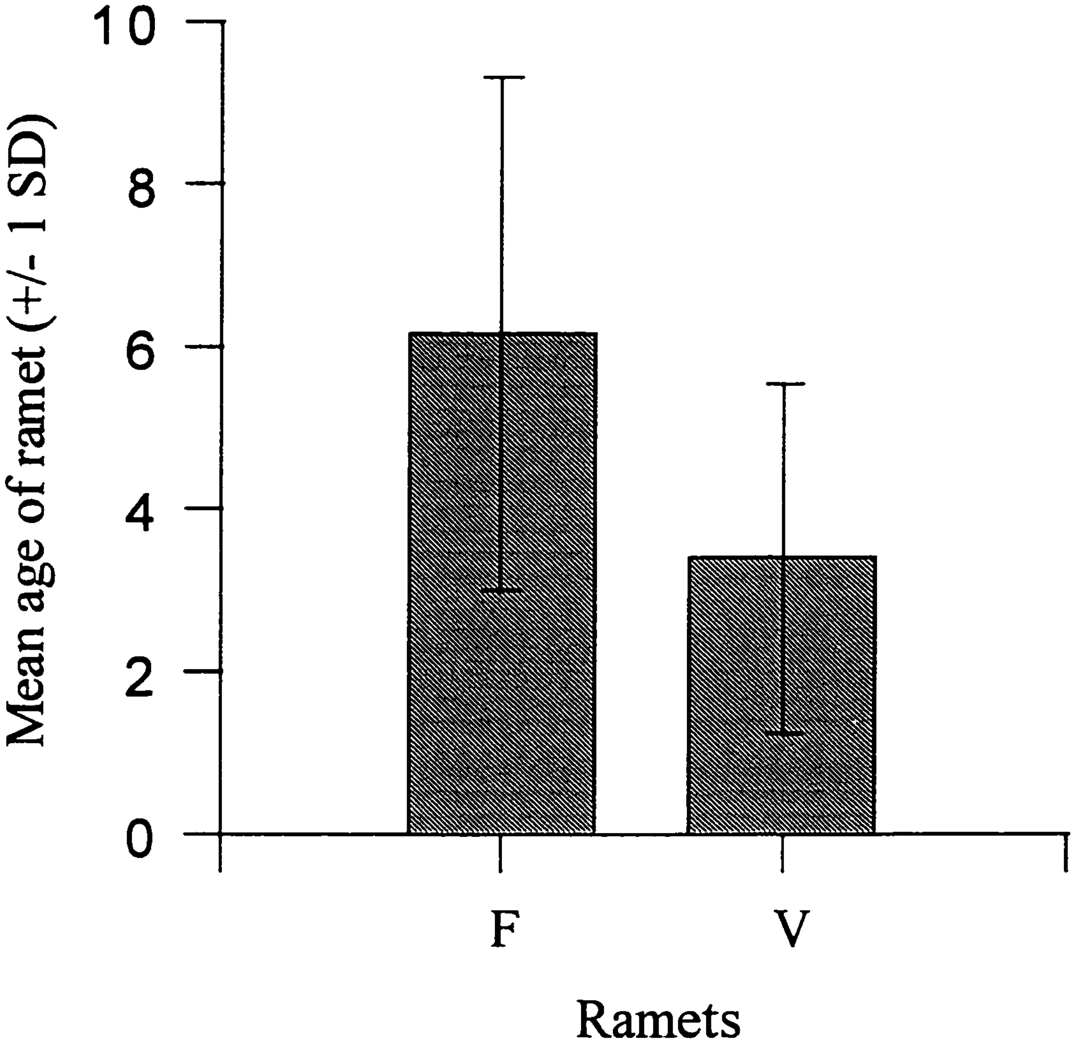

Figure 20. The mean age and standard deviation (SD) of flowering (F) and vegetative (V) ramets of mayflower.

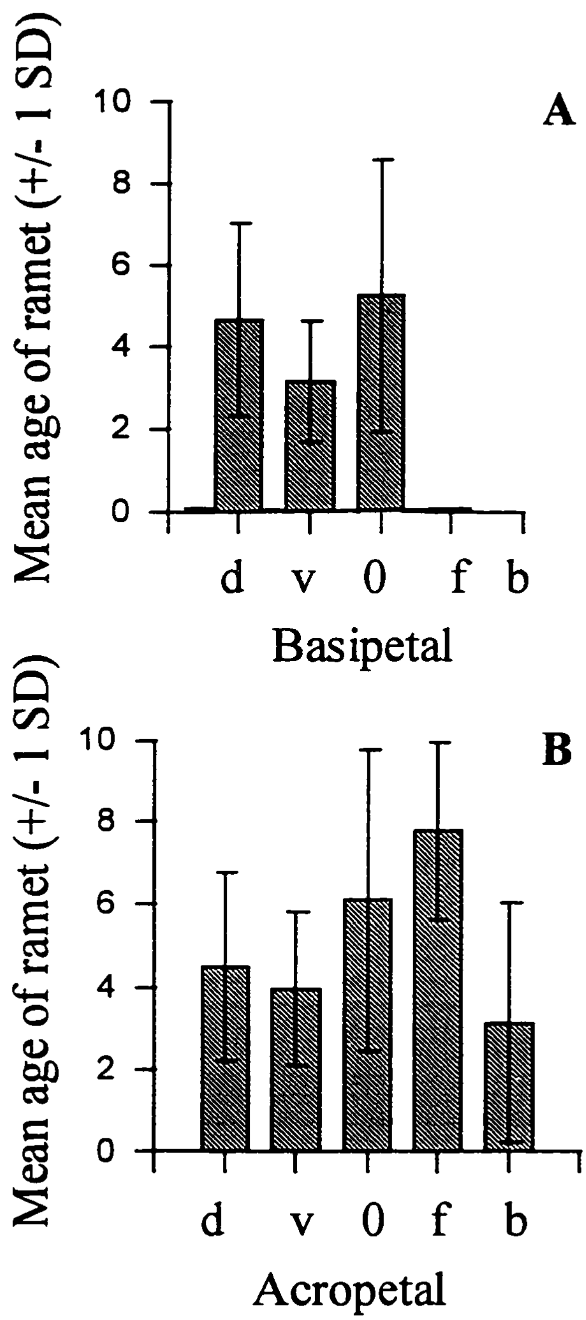

Figure 21. The mean age and standard deviation (SD) of ramets in each of the **A** basipetal and **B** acropetal contexts. d = dead, v = vegetative, 0 = no ramet, f = flowering, and b = new rhizome.

growing rhizome acropetal (p=0.040; Fig. 21B; Table 6). Ramets with no ramet acropetal were older than ramets with a vegetative ramet acropetal (p<0.01; Fig. 21B; Table 6).

Discussion

The percentage of vegetative ramets that produced a flowering bud was <10% (3 of 50 or 8 of 93). This was not unexpected since the percentage of flowering ramets in many local patches is typically under 10% (Ganger, unpublished data). Vegetative ramets that produced a flowering bud had significantly larger estimated leaf surface areas than vegetative ramets that produced a vegetative bud. Kana (1982) and Williams (1985) both found this to be the case with mayflower in their studies. It is not clear if a larger leaf surface area is needed to produce a flowering bud or if the developing flowering bud induces the production of a larger surface area leaf.

The estimated surface area of vegetative leaves was not related to the age of the ramets or to the number of years prior to/since flowering. The surface area of leaves in the pink lady's slipper orchid (Cypripedium acaule) has been shown to be related to the surface area of the leaf in the previous year (Primack and Hall 1990). In mayflower, Kana (1982) found a positive relationship (r^2=0.63) between the leaf size of a vegetative ramet in one year and the leaf size of the vegetative ramet in the following year. This is not inconsistent with the finding here that leaf surface area was not related to age. It appears that the leaf surface area of vegetative ramets is likely to be related to the previous year but that small ramets are likely to remain small and large ramets are likely to remain large.

Neither the local density of flowering ramets nor the nearest-flowering ramet distance was related to the number of seeds matured by flowering ramets. Had either of

Table 6. The mean age and standard deviation (SD) of mayflower ramets in each of the basipetal and acropetal contexts. The same lower case letters denote means that are not significantly different. Comparisons among basipetal contexts are made separately from acropetal contexts.

Context	n	Mean	SD
Dead ramet basipetal	12	4.67	2.35 a,b
Vegetative ramet basipetal	18	3.17	1.47 a
No ramet basipetal	70	5.27	3.32 b
Dead ramet acropetal	10	4.50	2.27 b,e
Vegetative ramet acropetal	38	3.97	1.84 b
No ramet acropetal	34	6.12	3.66 c,d,e
Flowering ramet acropetal	5	7.80	2.17 a,c
New rhizome acropetal	15	3.13	2.90 a,d

these variables been related to the number of seeds matured, then some aspect of the pollination biology of mayflower would have been suggested to be important. Lower nearest-neighbor distances typically correlate with greater a greater number of seeds matured for plants pollinated by insects that travel from nearest neighbor to nearest neighbor (Levin and Kerster 1968, 1969). A greater density of flowering ramets may mean a greater opportunity for pollination or a greater opportunity for competition for nutrients as well as pollinators (Heinrich 1979). Another aspect that may affect the number of seeds matured in mayflower is the genetic identity of the flowering ramets occurring nearby. Mayflower is self-incompatible (Worthen and Stiles 1986) and genetically similar flowering neighbors may lower the number of seeds matured by a chosen flowering ramets despite receiving pollen (Barrett and Thomson 1982, Handel 1985). The density of all ramets also had no effect on the number of seeds matured suggesting that competition for resources was not important in determining the number of seeds matured by flowering ramets. Flowering ramets do benefit from an attachment to other ramets (Chapter I) and any competition for resources that does occur may be offset by translocation of resources through the rhizome.

Reproductive history, which includes the number of times a ramet flowered during its lifetime and the number of years prior to or since flowering, was not related to the number of seeds matured by flowering ramets. It might be expected that ramets that waited longer to flower would have a larger store of resources available for flowering. Alternatively, ramets may wait for a critical level of resources before flowering—ramets in "poorer-quality" locations would wait longer to flower than ramets in "better-quality" locations. It might be expected that ramets that flowered for the first time would mature more seeds than ramets flowering for the nth time if there was a cost of previous flowering (see Chapter III). Alternatively, ramets in better quality locations might not experience this cost since resources can be replaced during the vegetative years of the ramet. The number of years prior to the first flowering of a ramet was significantly longer than the number of years between the first and second time flowering. Prior to the first flowering of a ramet, resources may be invested in the rhizome and in adjacent ramets. Ramets flowering for the second time are likely to have already borne this cost. Alternatively, ramets flowering for a second time may find themselves in "better" locations than ramets flowering for only the first time.

Only 15% of the flowering ramets existed with a vegetative or flowering ramet both basipetal and acropetal. The flowering ramets that were physiologically isolated (49.1%) were not as successful as those with a vegetative ramet acropetal. Whether the success of ramets with a vegetative ramet acropetal is due to subsidization of the flowering ramet by the vegetative ramet or overall greater health is not known. However it is surprising that the most numerous class of flowering ramets was the one with the lowest number of seeds matured. In these isolated ramets, the number of seeds matured was related to the total amount of rhizome available. This suggests that the rhizome is providing resources for flowering ramets to mature seeds.

Eriksson and Jerling (1990) categorize plants as either "genet splitters," in which the duration of genet integration is shorter than one year, or "integrated genets," in which integration is much longer. They list Rubus saxatalis, Fragaria moschata, Trientalis borealis, Potentilla reptans, Potentilla anserina, Fragaria vesca, Aster acuminatus, and Medeola virginiana as genet splitters and list Linnaea boeralis, Lycopodium clavatum, Pteridium aquilinum, Maianthemum canadense, Cornus canadensis, Gaultheria procumbens, Clintonia borealis, and Oxalis montana as integrated genets. Their classification is based in part on the premise that maintaining integration between widely dispersed ramets would be more expensive than maintaining integration between close ramets. In fact Eriksson and Jerling (1990) state that overall, genet splitters tend to have longer between ramet spacings than integrated genets.

The term integrated genet may be somewhat of a misnomer and in fact ramets of both C. borealis (Ashmun and Pitelka 1982) and M. canadense (Silva 1978 cited in Pitelka and Ashmun 1985, Chapter I) have been shown to be physiologically independent the majority of the time, but have the ability to integrate under periods of stress such as defoliation, shading, or increased seed maturation. This may mean that ramets experimentally demonstrated to be physiologically independent may actually be integrated if they do not display independent mortalities in conditions of prolonged stress since initially, the stress may be buffered by the utilization of locally stored carbohydrate (Marshall 1990). Even with the "break up" of the genet through the disintegration of the connections between ramets, ramets may still experience a subsidy since the resources stored in the local rhizome may have been translocated there by the genet.

Growing rhizomes develop with subsidization by the genet (Williams 1964, Rogan and Smith 1974, Ryle et al. 1981, Noble and Marshall 1983) and may serve a variety of functions such as 1) a storage organ for carbohydrates (Ashmun et al 1982), 2) the uptake of water and nutrients and translocation to young ramets (Marshall 1990), 3) conservation and recycling of nutrients within a genet at a nutrient poor site (Callaghan 1980), and 4) keeping a bud bank (Ashmun et al. 1982, Fagerström 1992). The rhizome in mayflower may also be thought of as a method for the placement ramets in space and subsidy of them through the early years prior to their flowering. As the "placed" ramet becomes more independent it may begin subsidizing its own acropetal ramet and as the "old" connection decays this ramet may flower.

Flowering ramets were older than vegetative ramets and this is likely to do with the fact that no ramet flowered in its first or second year and many ramets delayed flowering for a longer period of time (Chapter I). The ramets with a basipetal vegetative ramet were younger than those with no ramet basipetal. Ramets with a flowering ramet acropetal were older than ramets with a growing rhizome acropetal, a vegetative ramet acropetal, or a dead ramet acropetal. Ramets with a vegetative ramet acropetal were younger than ramets with no ramet (decayed rhizome) acropetal. There was no difference in the ages of ramets with a flowering ramet acropetal and ramets with no ramet acropetal. The basipetal ramet is likely to exist as a vegetative ramet initially and a consequence of the aging of the focal ramet is the reduction or cessation of integration, either through disintegration of the connection between the focal and basipetal ramet or the mortality of the basipetal ramet. The acropetal ramet appears to follow one of two possible developmental pathways. The acropetal ramet begins as a growing rhizome, develops into a vegetative ramet, and then either the acropetal ramet develops into a flowering ramet or the potential for integration is reduced or ceases as the acropetal ramet dies or the rhizome between the focal and acropetal ramet dies.

Despite the fact that most flowering ramets existed as isolated ramets the variables that related directly to the ramet, such as ramet age, reproductive history, local density of flowering ramets, and nearest flowering ramet distance did not explain any of the variation in the number of seeds matured among flowering ramets. However variables related to the genet (such as context and amount of rhizome) did explain some of the variation in the number of seeds matured. Consequently, it appears that, for mayflower, a ramet-based approach to demography would not be as predictive as an approach that incorporated some aspects of the genet.

CHAPTER V

THE ROLES OF RAMET CONTEXT AND SHADE ON THE CURRENT SEXUAL REPRODUCTION OF A CLONAL HERB

Abstract

A manipulative field experiment was undertaken to examine the role of shading, ramet context, and pollen addition on the number of seeds matured by the ramets of Canada mayflower (Maianthemum canadense var. canadense Desf.). Flowering ramets that were shaded matured more seeds than unshaded flowering ramets. Flowering ramets that were overpollinated matured comparable numbers of seeds to those flowering ramets that were open pollinated. Flowering ramets connected to another flowering ramet, either basipetal or acropetal, matured more seeds than flowering ramets in other contexts with the exception of flowering ramets with either no ramet or a dead ramet basipetal and a vegetative ramet acropetal. Flowering ramets and vegetative ramets differed in the distribution of ramets among contexts indicating that these ramets are not only anatomically different but differ as well in their potential for physiological integration. Flowering ramets were older than vegetative ramets. Ramets in the 'no ramet' or 'dead ramet both acropetal and basipetal' contexts were consistently older than ramets in other contexts with the exception of ramets connected to a flowering ramet.

Introduction

Plant genets are often composed of a number of ramets connected by rhizomes or stolons. To the extent these ramets are physiologically integrated, ramet survivorship and reproductive success may be different from that of isolated ramets (Pitelka and Ashmun 1985). Though connected, individual ramets may be quite independent, but integrate during periods of stress (Pitelka and Ashmun 1985, Marshall 1990). Ramets of Fragaria chiloensis were shown to translocate resources to shaded and water stressed sister ramets, increasing their survivorship—isolated ramets experienced very high mortalities (Alpert and Mooney 1986). Ramets of Clintonia borealis were found to translocate resources to defoliated sister ramets (Ashmun, Thomas, and Pitelka 1982). Ramets of Ambrosia psilostachya were found to translocate resources to sister ramets in high salinity locations (Salzman and Parker 1985).

Ramets of Solidago canadensis have been demonstrated to translocate resources to shaded, sister ramets (Hartnett and Bazzaz 1983). Translocation in S. canadensis appeared to last seven weeks, after which time unshaded ramets ceased to subsidize shaded ramets. Based on these results, the extent of translocation and the length of time that ramets remain connected (potential for integration) is thought to be under selective pressure (Marshall 1990). As such, the "connectedness" of ramets may differ among genets and among locations. In Salvinia molesta, genets remain connected longer in "infertile" locations than in "fertile" locations (Room 1983). This is evidence for the theory that young ramets develop through subsidy and then eventually become independent and subsidize their own daughter ramets (Marshall 1990). The length of time that a ramet remains in each phase may be related to its own resource state. Daughter ramets in low resource locations may remain dependent longer, as they slowly accumulate resources.

For understory herbs, such as Canada mayflower (Maianthemum canadense var. canadense Desf.; Gleason and Cronquist 1991), light may be an important "resource" and the light regime experienced by mayflower may differ dramatically depending on neighbor canopy species. A deciduous canopy may allow for high light levels during the early growth of mayflower (prior to leafing out of the trees) and then low light levels during mayflower's fruiting season. A coniferous canopy may present a more uniform light regime throughout the season (Ashmun and Pitelka 1984).

There is evidence that shading has a negative effect on the number of seeds matured by mayflower. In a pilot experiment (Ganger, unpublished data), flowering ramets that were shaded and connected to a basipetal vegetative ramet matured fewer seeds than flowering ramets that were not shaded and connected to a basipetal vegetative ramet. However, flowering ramets that were not shaded and connected to a shaded, vegetative ramet matured more seeds than flowering ramets connected to an unshaded, vegetative ramet.

The tentative conclusion is that mayflower ramets compete for resources stored in a common rhizome and that the unshaded ramet is benefitting at the expense of the shaded ramet. This type of an experiment deserves to be repeated with a larger sample size and more common contexts.

In Chapter IV, the number of seeds matured by flowering ramets was found to be related to their context, i.e., the identity of the ramets directly basipetal and acropetal on the same rhizome system. These ramets were collected over a large area (3 hectares) and therefore little is known about the relationships, if any, between specific locations and ramet context. For example, are specific contexts more prevalent in specific locations?

Since shading, context (Chapter IV), and pollen addition (Chapters I and III) have been shown to affect the number of seeds matured, these three factors will be considered together in a manipulative field experiment that addresses the following questions: Was the number of seeds matured by mayflower pollen limited? Did ramets that were shaded mature fewer seeds than unshaded ramets? Did flowering ramets with different contexts differ in the number of seeds that they matured? Did vegetative and flowering ramets differ in their contexts? Did vegetative and flowering ramets differ in age? Did ramet contexts differ with location?

Methods

In the spring of 1997, seven locations were selected within the University of New Hampshire woodlands, Durham, NH based on the presence of mayflower. Within each location 30 flowering buds were selected. Ten buds were randomly assigned to each of three treatments: shaded, overpollinated; unshaded, overpollinated; and unshaded, open pollinated. The shaded treatment consisted of a shadecloth hood that was placed over the flower ramet from the time that the ramet reached its maximum height (prior to flowering) until the end of the fruiting season. These hoods reduced light levels by 50%.

In May, when the ramets began to flower, all flowers of ramets in the shaded, overpollinated and the unshaded, overpollinated treatments received an overabundance of exogenous pollen each day for the life of the flowers. Pollen was collected immediately prior to use from 10–20 flowering ramets not more than 20 m away and applied to the ramets with a toothpick. Flowering ramets in the unshaded, open-pollinated treatment were unmanipulated and presumably received natural levels of pollination.

At the end of the fruiting season, all of the flowering ramets were excavated to include any ramets directly basipetal and acropetal on the same rhizome, as well as any ramets that originated from this rhizome segment, and taken to the laboratory. In the laboratory, the number of fruits and seeds matured by each ramet was determined. The age and reproductive history (whether the ramet was vegetative or flowering in each of its previous years) of each experimental ramet along with the identity, age, and reproductive history of any basipetal or acropetal ramets was determined.

Also at the end of the fruiting season in locations adjacent to but not contiguous with the flowering-ramet locations, 20 vegetative ramets were haphazardly selected, excavated to include any ramets directly acropetal and basipetal on the same rhizome as well as any ramets that originated on this rhizome segment, and taken to the laboratory. In the laboratory, similar data was collected for these ramets as was collected for the flowering ramets.

A two-way analysis of variance (ANOVA) was used to assess differences in the number of seeds matured by flowering ramets. The first factor was "flowering" (shaded, overpollinated; unshaded, overpollinated; and unshaded, open pollinated) and the second factor was "context." Normally a variable such as context would be treated as a covariate, however in this case it was treated as a classification factor following Winer et al. (1991). Both factors were fixed. Site was also included as a blocking factor (fixed) and as such none of the interactions with site were calculated. The interaction of flowering and context was not able to be included due to a problem with the distribution of contexts among treatments. Two a priori contrasts were of interest. In order to determine if the number of seeds matured was pollen limited, the number of seeds matured by the unshaded, overpollinated ramets was compared to the number of seeds matured by the unshaded, open-pollinated ramets. In order to determine if there was an effect of shading, the number of seeds matured by shaded, overpollinated ramets was compared to the number of seeds matured by the unshaded, overpollinated ramets. The second contrast involved a resource effect and therefore the amount of pollen was controlled for, with each treatment receiving an overabundance of pollen.

In order to determine if flowering and vegetative ramets differed with respect to their contexts, a two-way Chi-square (χ^2) test of independence was performed (Wilkinson 1991).

In order to determine if aspects of the vegetative and flowering ramets differed with respect to their ages, a three-way ANOVA was performed with age of the ramet as the dependent variable. The three factors were 1) ramet type (flowering or vegetative), 2) site (each of the seven locations), and 3) ramet context. Each of the two-way interactions was considered; however, the three-way interaction was not included due to a problem with the distribution of contexts among ramets and among sites. Each of the three factors was fixed. Ramet context was considered a classification factor following Winer et al. (1991). Site in this case was also considered as fixed since sites were not chosen randomly but based instead on their high densities of flowering ramets.

Results

The number of seeds matured was generally low. Thirty of sixty-four ramets (47.9%) in the shaded, overpollinated treatment matured seed. Only 5 of 61 ramets (8.2%) in the unshaded, overpollinated treatments matured seed, and 3 of 53 ramets (5.6%) matured seed in the unshaded, open-pollinated treatment. Thirty-one ramets were lost or could not be accurately aged or their reproductive histories determined and therefore were not included in the analyses.

There was no difference in the number of seeds matured by flowering ramets among sites ($F_{6,160,0.05}$=1.649, p=0.137). There was a significant difference in the number of seeds matured by ramets among treatments ($F_{2,160,0.05}$=8.834, p<0.001). The first a priori contrast revealed that the number of seeds matured by mayflower was not pollen limited ($F_{1,160,0.05}$=0.0542, p=0.816; Fig. 22A). The second a priori contrast found that shaded, overpollinated ramets matured more seeds than the unshaded, overpollinated ramets ($F_{1,160,0.05}$=14.260, p<0.001; Fig. 22B). There was a significant difference in the number of seeds matured by flowering ramets in different contexts ($F_{4,160,0.05}$=3.931, p<0.005). Flowering ramets that had another flowering ramet basipetal or acropetal matured more seeds than the other contexts with the exception of flowering ramets with either a dead ramet or no ramet basipetal and a vegetative ramet acropetal (Fig. 23; Table 7).

Flowering ramets and vegetative ramets were represented in each of the six contexts (Table 8). However, a two-way Chi-square test of independence found a significant difference between flowering ramets and vegetative ramets with respect to their

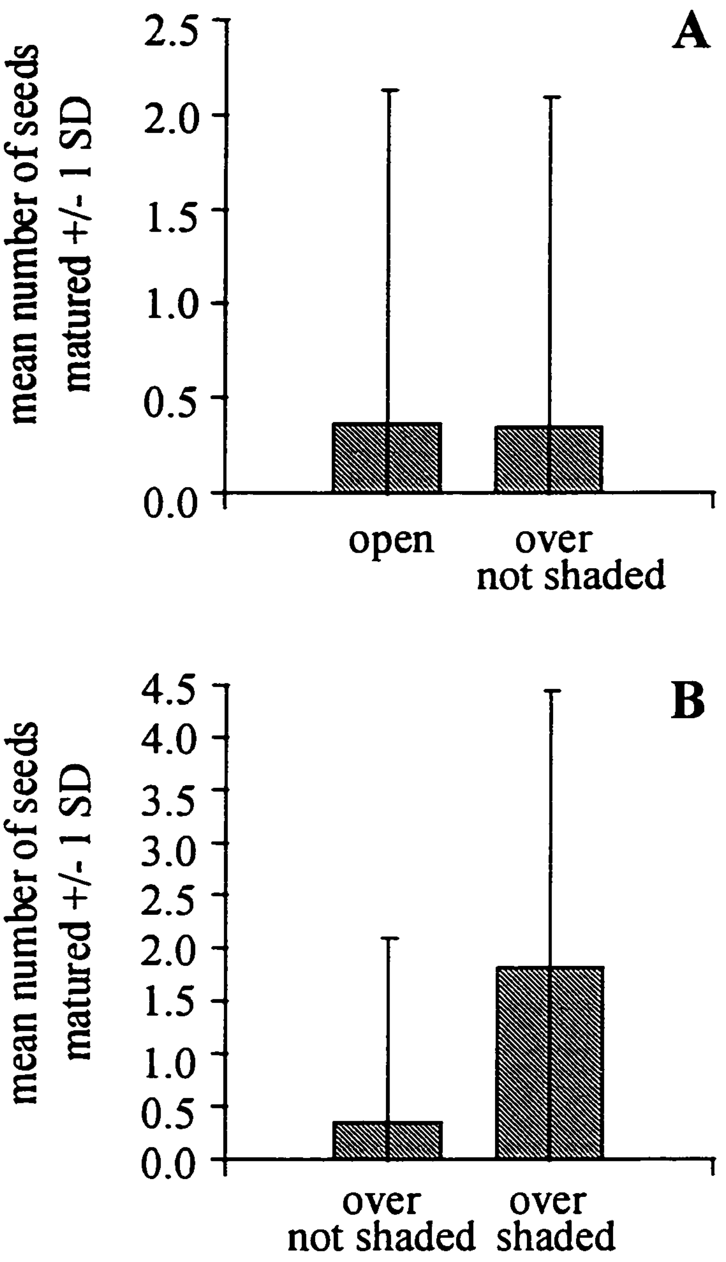

Figure 22. Mean number and standard deviation (SD) of the number of seeds matured by flowering ramets in the **A** unshaded, open-pollinated (open) and unshaded, overpollinated (over) treatments and **B** unshaded, overpollinated (over) and shaded, overpollinated (over) treatments.

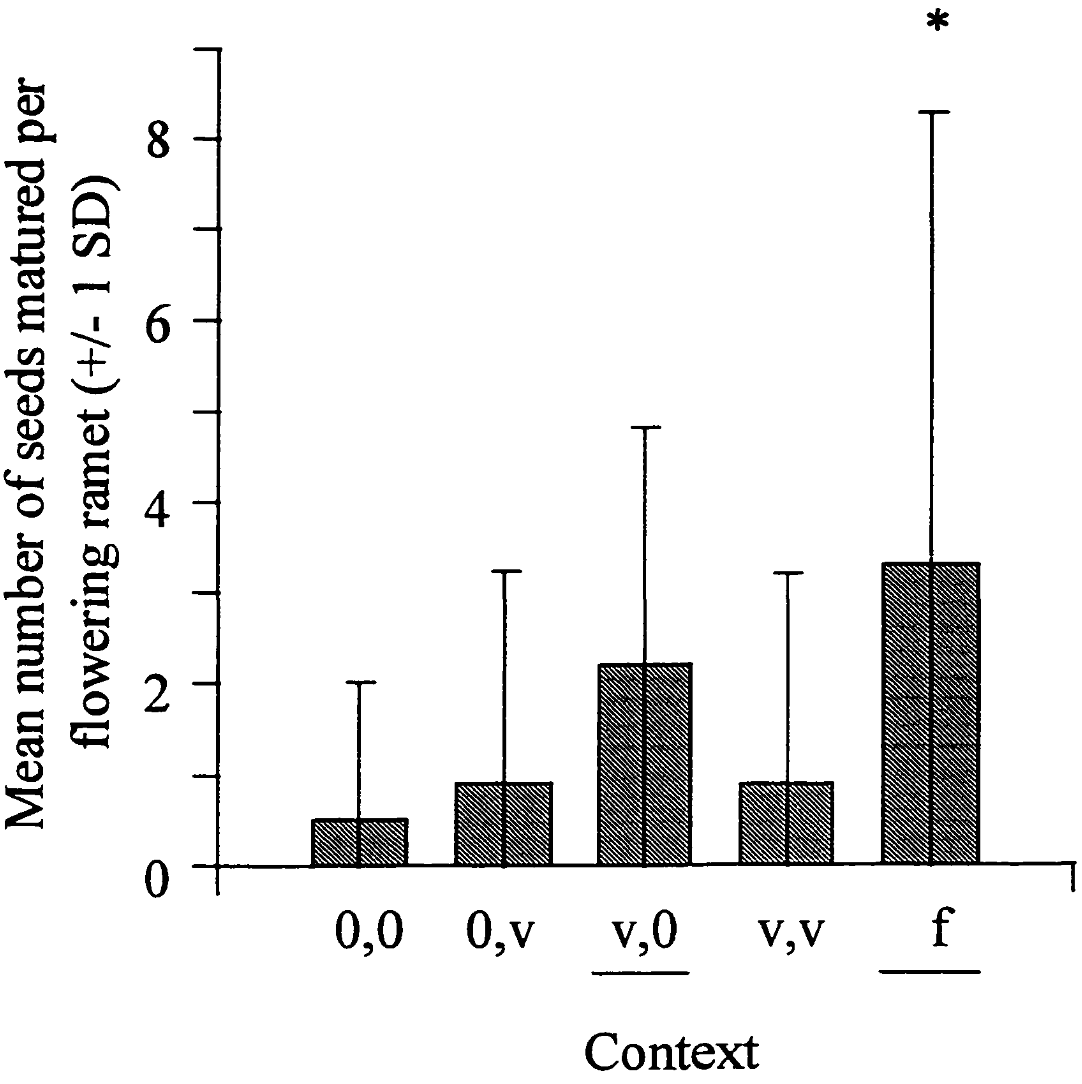

Figure 23. Mean number and standard deviation (SD) of the number of seeds matured by flowering ramets in each of the ramet contexts. 0 = dead ramet or no ramet, v = vegetative ramet, and f = flowering ramet.

Table 7. The mean number and standard deviation (SD) of seeds matured by flowering ramets of mayflower in each of the contexts. Small case letters denote means that are similar.

Context	n	Mean	SD
0,0	102	0.51	1.50 a
0,v	42	0.91	2.33 a
v,0	10	2.20	2.62 a,b
v,v	9	0.89	2.32 a
f	10	3.30	4.97 b

Table 8. The number of flowering and vegetative ramets in each of the contexts. Numbers in parentheses indicate the percentage of ramets within specific contexts. 0 = dead ramet or no ramet, v = vegetative ramet, f = flowering ramet, b = growing rhizome.

Context	Flowering	Vegetative
0,0	102 (53.4%)	34 (25.6%)
0,v	46 (24.1%)	38 (28.6%)
v,0	10 (5.2%)	10 (7.5%)
v,v	10 (5.2%)	22 (16.5%)
f	18 (9.4%)	6 (4.5%)
b	5 (2.6%)	23 (17.3%)
Total n	191	133

distribution among contexts ($\chi^2_{5,0.05}$=2867.21, $p<0.001$). Fifty-three percent of flowering ramets existed with no ramet or a dead ramet both acropetal and basipetal while 25.6% of vegetative ramets were found to have the same context. Only 2.6% of flowering ramets were found to have a growing rhizome acropetal while 17.3% of vegetative ramets were found in this context.

Flowering ramets were found to be on average 5.65 years old (SD=1.96) and vegetative ramets ranged from 1 to 10 years old (mean=3.97, SD=1.62). Flowering ramets were significantly older than vegetative ramets ($F_{1,252,0.05}$=8.840, $p<0.005$; Fig. 24). Overall, ramets differed in ages among the seven sites ($F_{6,252,0.05}$=2.502, $p<0.05$) and among contexts ($F_{5,252,0.05}$=4.057, $p<0.005$; Fig. 25, Table 9). Ramets with a dead ramet or no ramet both basipetal and acropetal were older than ramets in the other contexts except for ramets with a flowering ramet acropetal or basipetal. The site*ramet type interaction was significant as well ($F_{6,252,0.05}$=3.456, $p<0.005$; Fig. 26) making an interpretation of the two main effects, site and ramet type, more difficult. The ages of ramets in specific contexts did not vary among sites (site*context interaction; $F_{30,252,0.05}$=0.651, p=0.952). The ages of ramets in specific contexts did not differ whether they were vegetative or flowering ramets (ramet type*context interaction; $F_{5,252,0.05}$=0.835, p=0.526).

Discussion

The number of seeds matured overall in 1997 was very low and in fact most ramets did not mature seeds. The number of seeds matured by mayflower at these sites was not pollen limited, as it was in previous years, but was likely resource limited. Shaded,

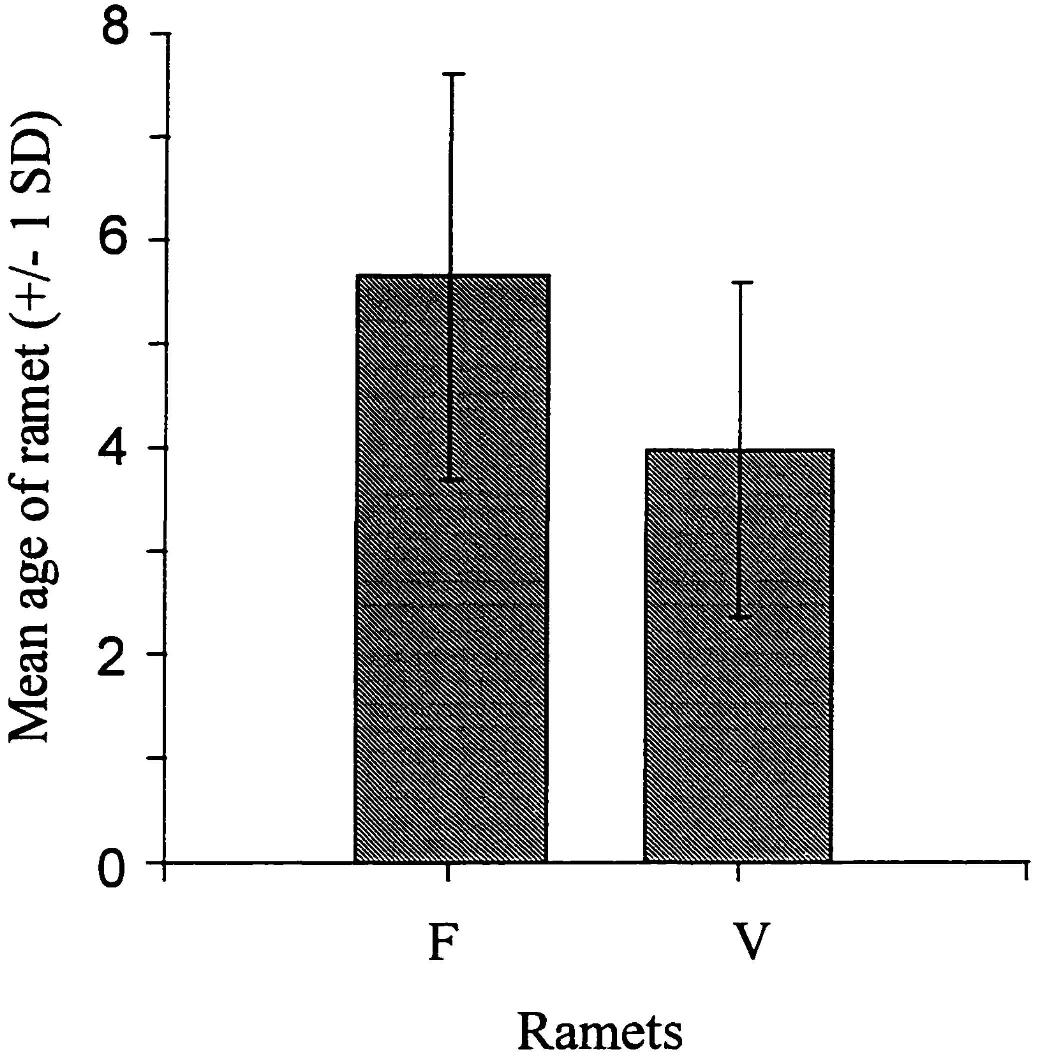

Figure 24. Mean age and standard deviation (SD) of flowering (F) and vegetative (V) ramets.

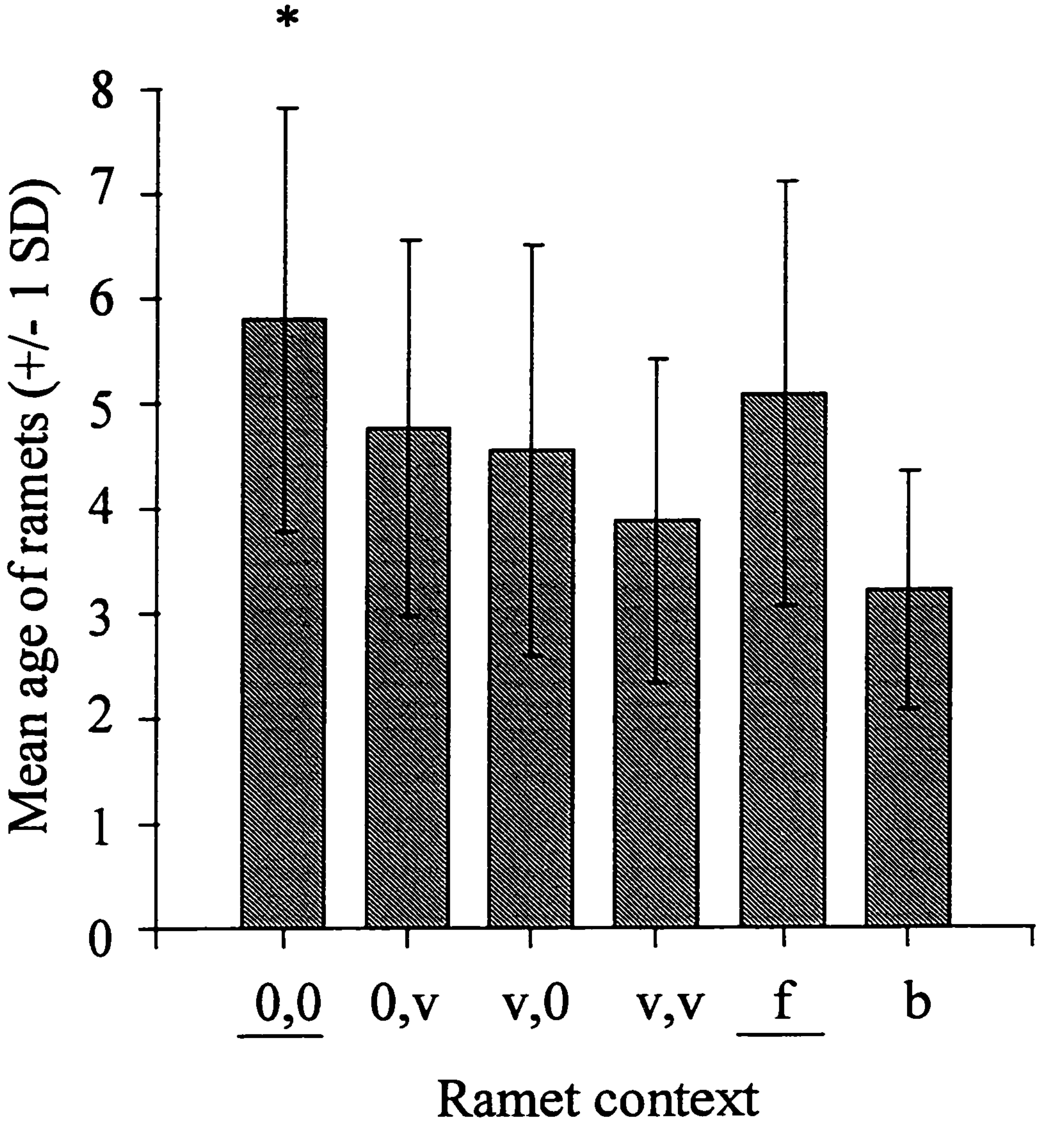

Figure 25. Mean age and standard deviation (SD) of mayflower ramets in each context. 0 = dead ramet or no ramet, v = vegetative ramet, f = flowering ramet.

Table 9. The mean age and standard deviation (SD) of ramets in each of the contexts. Lower case letters denote means that are not significantly different. 0 = dead ramet or no ramet, v = vegetative ramet, f = flowering ramet, b = growing rhizome

Context	n	Mean	SD
0,0	136	5.80	2.02 a
0,v	84	4.76	1.79 b
v,0	20	4.55	1.96 b
v,v	32	3.88	1.54 b
f	24	5.08	2.02 a,b
b	28	3.21	1.13 b

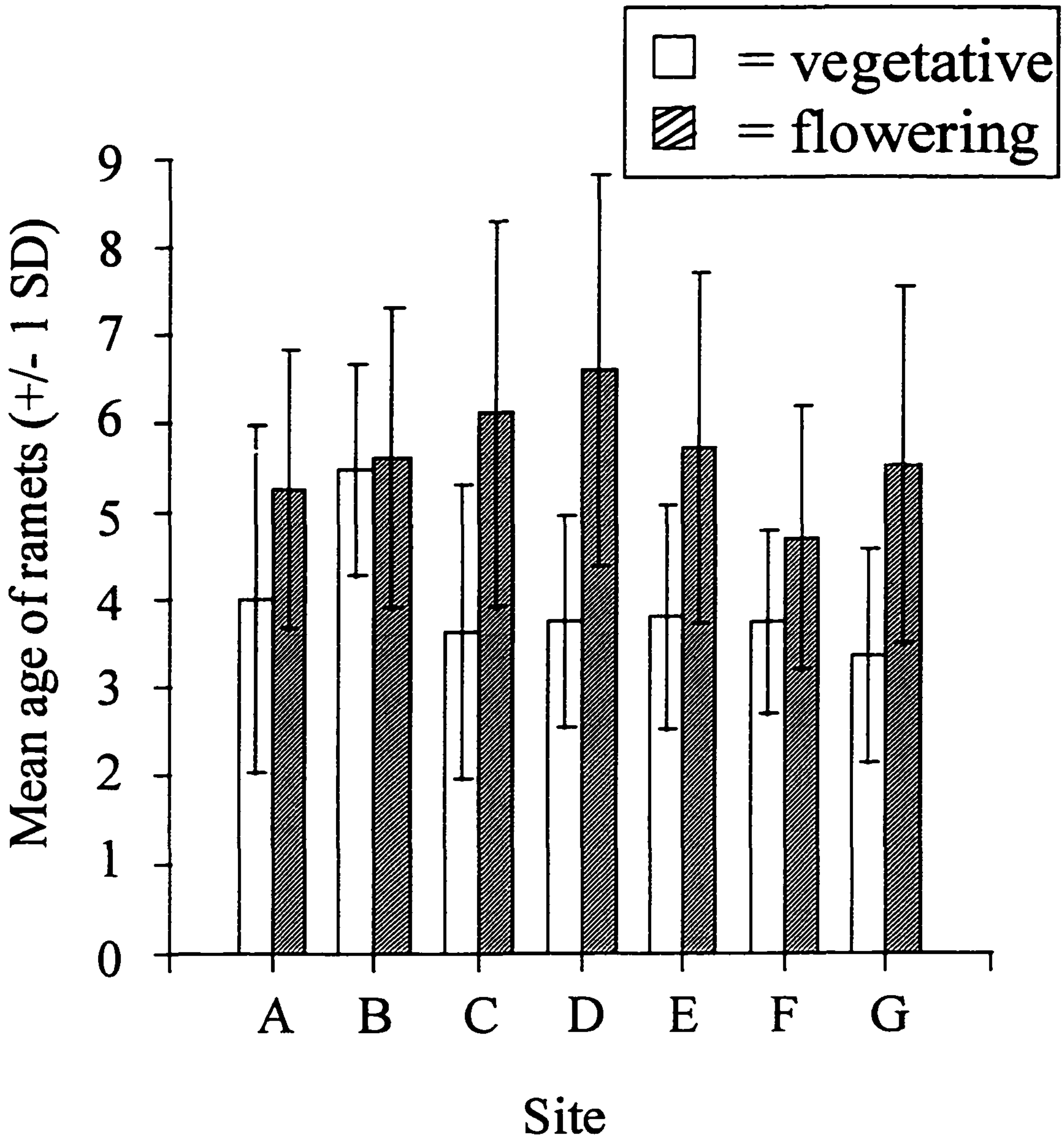

Figure 26. Mean age and standard deviation (SD) of vegetative and flowering ramets at each of the seven sites.

overpollinated ramets were probably not resource limited to the same extent that unshaded overpollinated ramets were, as the former matured more seeds. There are at least two hypotheses to explain this: 1) unshaded ramets were stressed to a greater extent than shaded ramets or 2) shaded ramets were subsidized by other ramets on the same rhizome.

Data from 1994, 1995, 1996, and 1997 may provide insights into the first hypothesis. The number of seeds matured by mayflower was found to be pollen limited in the University of New Hampshire woodlands in three of four years (Fig. 27). It appears that in 1997 the number of seeds matured by overpollinated ramets was depressed instead of the alternative, which would be an increase in the number of seeds matured by open-pollinated ramets. This suggests that the number of seeds matured by ramets was resource limited in 1997 and that this acted to reduce the number of seeds matured despite adequate pollination. It is possible to infer whether ramets received adequate pollination and to determine if resource limitation was experienced early in the season prior to flowering or whether resource limitation was experienced throughout the fruiting season. An initial count of fruits was made for each of three years (1994, 1995, and 1997) as well as a final count. The initial count was made 10–14 days after the cessation of hand pollinations. The number of fruits was used as a variable as seed number could not be accurately estimated until much later in the fruiting season.

For 1994, 1995, and 1997 the mean number of fruits present during the first and final censuses was determined and plotted (Fig. 28). If the mean number of fruits present during the first census for ramets in 1997 was similar to that of ramets in 1994 and 1995, then severe resource limitation was not likely experienced prior to flowering. If resource limitation was experienced throughout the fruiting season, then the mean number of fruits

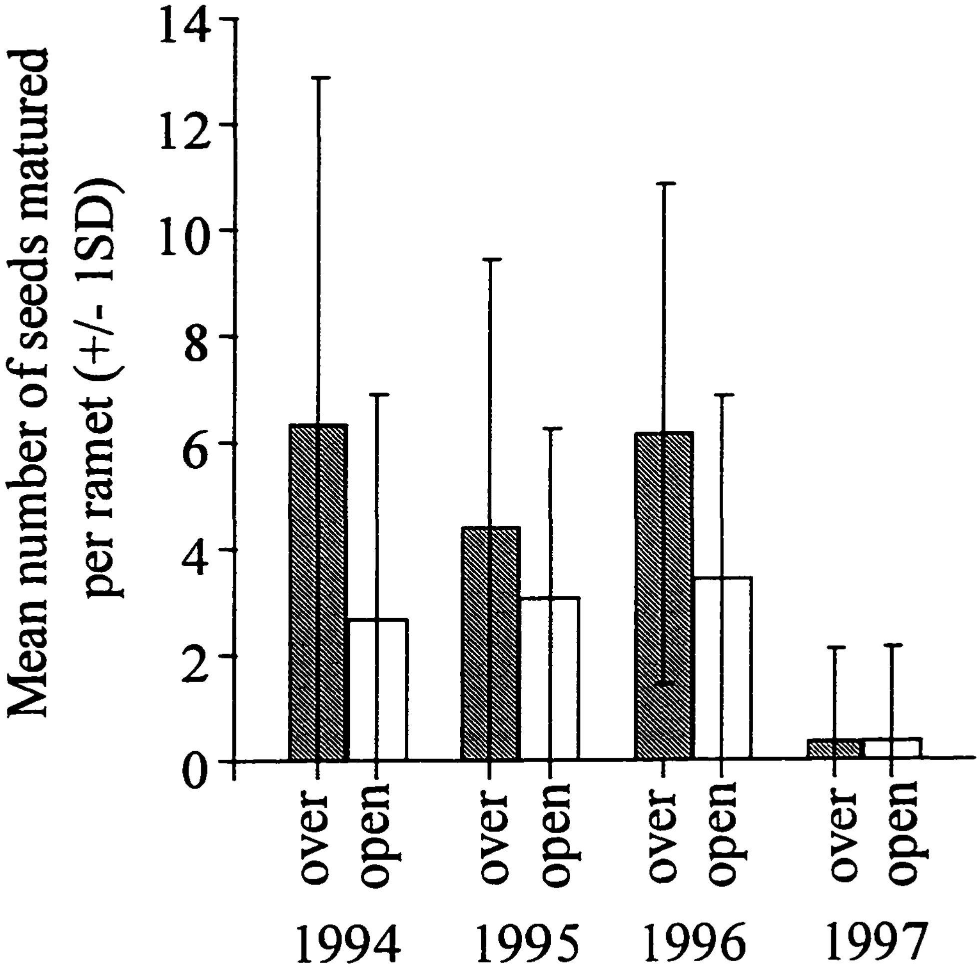

Figure 27. Mean number and standard deviation (SD)of seeds matured by over-pollinated (over) and open-pollinated (open) ramets in each of the four years.

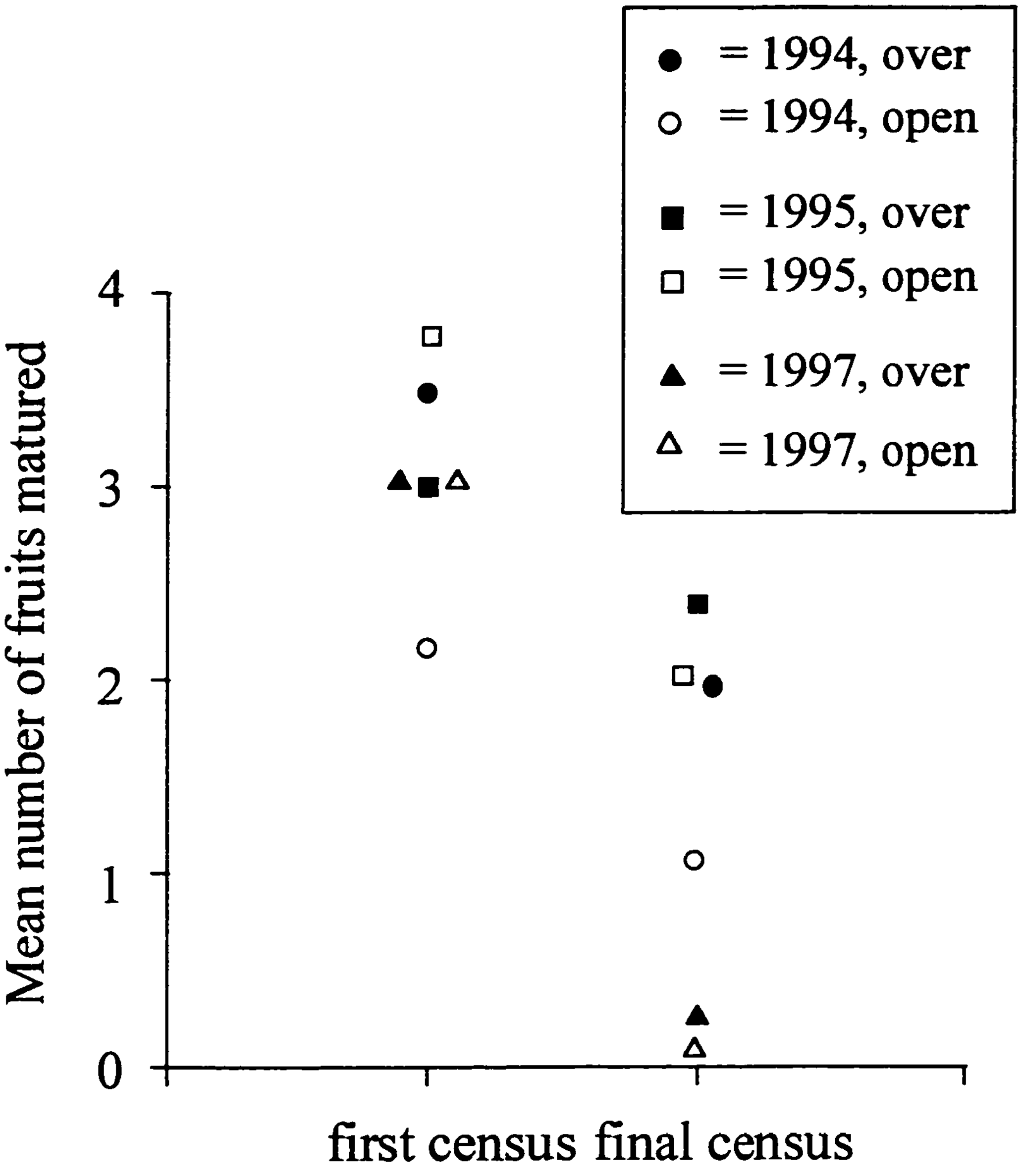

Figure 28. The mean number of fruits present on overpollinated (over) and open-pollinated (open) ramets at the first and final censusses.

present during the final census of ramets in 1997 would be expected to be much lower. This appears to be exactly what occurred. In fact, in 1997, there was very little rainfall in the month following mayflower pollination: only 0.7 inches of rain fell in June 1997. In June 1994, 1.94 inches of rain fell, in June 1995, 1.36 inches of rain fell, and in June of 1996, 3.06 inches of rain fell (National Oceanic and Atmospheric Administration 1994, 1995, 1996, 1997). The year 1997 may be considered a drought year for mayflower.

It appears that in a given year, at the scale of these experiments, the number of seeds matured by ramets is a function of pollen availability and quality. This pattern is likely to be modified by the resource-state of individual ramets, but not to the extent that the overall pollen limitation trend disappears. It is only with a large-scale event like a drought that resource limitation overwhelms the effect of pollen availability and quality.

This is not to suggest that in an average year resources are not important, only that the scale of resource limitation is likely to be with individual ramets and in an average year the pattern of pollen limitation is still evident overall. In fact, these experiments have shown that the number of seeds matured by individual ramets is influenced by whether the rhizome was left intact or severed. In 1994 and 1995 ramets that were overpollinated and severed from the genet matured fewer seeds and fruits than overpollinated ramets left connected to the genet. The inference from this result is that with high seed and fruit set additional resources are being used from outside those associated with the experimental ramet. Resources are likely being translocated from local rhizome; however, resources may also be coming from adjacent ramets on the same rhizome. Ramets that were open pollinated matured similar numbers of seeds and fruits whether their rhizomes were left intact or severed.

It is also possible that shaded, overpollinated ramets were subsidized by other ramets on the same rhizome (hypothesis 2). This hypothesis is not as likely to be correct since many of these shaded, overpollinated ramets existed as isolated ramets: a dead ramet or no ramet basipetal or acropetal (41.5%).

In 1997, context was shown to be related to the number of seeds matured. This was also the case in 1996 (Chapter IV); however, there were differences in the way in which data were collected in these two years. In 1996, 53 flowering ramets were haphazardly collected from within a three hectare area of the University of New Hampshire woodlands. Each ramet was collected from a site with potentially different light regimes and community structures. For example, ramets were collected from locations beneath deciduous, white pine (Pinus strobus), and eastern hemlock (Tsuga canadensis) canopies. In 1997, the 210 flowering ramets used in the experiment were located under canopies dominated by white pine. The ramets came from only seven canopies rather than from the range of canopies in 1996. Despite these differences it is useful to consider these data from different years to determine if some trends were repeated across years.

In both years (Fig. 29) ramets that had a dead ramet or no ramet acropetal and basipetal matured fewer seeds. This result was obtained despite the overall depression in the number of seeds matured by ramets in 1997.

Flowering and vegetative ramets differed from one another with respect to their contexts. This is perhaps surprising as flowering ramets and vegetative ramets are not distinct populations. Flowering ramets will have been vegetative ramets in the previous year and, if they survive, will be vegetative ramets in the following year. Over 80% of the

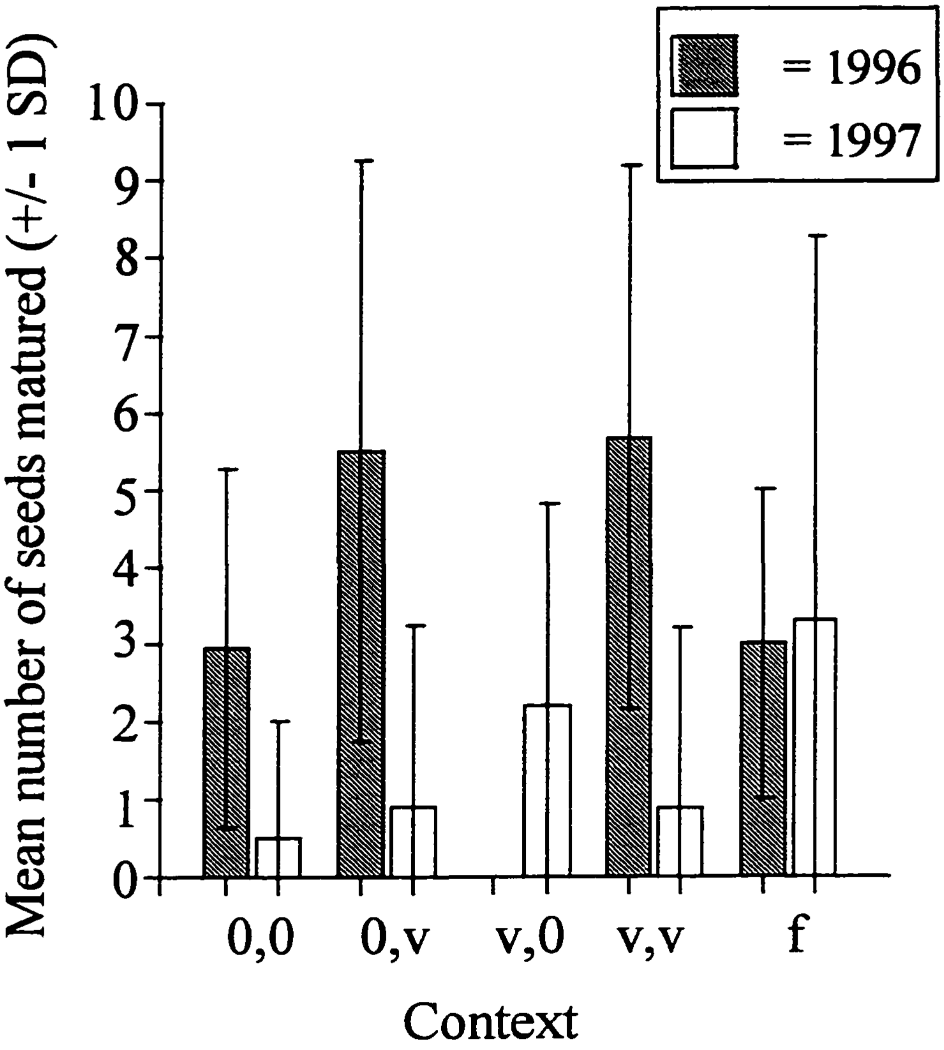

Figure 29. Mean number and standard deviation (SD) of the number of seeds matured by flowering ramets in each context for 1996 and 1997.

ramets that were found to be producing a new acropetal ramet were vegetative while only 17.9% were flowering ramets. In addition, vegetative ramets are more likely to be connected to other ramets (74.4%) than flowering ramets which are more likely to exist as isolated ramets (53.4%).

The pattern of age with respect to context as well as the frequency of contexts for vegetative and flowering ramets allows a first attempt at reconstructing ramet and genet development. A new acropetal ramet is produced on average when the ramet is 3.2 years old (SD=1.13). Isolated ramets average 5.8 years in age (SD=2.02) and are significantly older than ramets in the other contexts with the exception of those with a flowering ramet basipetal or acropetal. The differences in flowering and vegetative ramet contexts are likely due to the difference in the ages of flowering and vegetative ramets. Flowering ramets tend to be older than vegetative ramets.

Based on the ages of ramets in the collections, ramets did appear to pass through predictable stages. A new rhizome/bud is produced by a vegetative ramet that is about 3.2 years old (2.4 years old based on the 1996 data). This new ramet (focal ramet) will then remain vegetative for approximately 3.2 years (2.4 years based on the 1996 data), when it will then produce a new rhizome/bud. The focal and acropetal ramets would then pass through their own stages probably independent of one another. The focal ramet is vegetative and then that ramet dies or the rhizome between the two decays. The acropetal ramet is vegetative and then becomes a flowering ramet (in only a few cases) or the rhizome between the two (focal and acropetal ramets) decays (in most cases). It appears that fragmentation (the breaking apart of ramets from each other through rhizome decay) is very common in mayflower and that most ramets are likely to flower without the possibility of aid from other ramets. Furthermore, the likelihood that two ramets on the same rhizome will be flowering concurrently is very low.

The extent of the differences in ages between vegetative and flowering ramets differed among sites (Fig. 26). The interaction of site and ramet type probably occurred because vegetative and flowering ramets at site B were similar in age. The recruitment of new acropetal ramets may be limited in this site and therefore the long term persistence of mayflower there may be in question. In each of the other sites, vegetative ramets are similar to each other and consistently younger than flowering ramets. The average age of flowering ramets, however, varies and this is likely due to the length of time that ramets wait to flower. For example, ramets in site F may be flowering earlier than ramets in site D. In fact the ramets at site D were found to be younger than ramets at each of the other sites except site A.

It is difficult to place these results within the framework of clonal plant research in general for a number of reasons. First, not all clonal plants have distinct flowering and vegetative ramets, although many understory plants do—mayapple (Podophyllum peltatum; Sohn and Policansky 1977), wild sarsparilla (Aralia nudicaulis; Barrett and Thomson 1982). Second, aging of ramets may be difficult—including those of some understory plants such as Clintonia borealis (Pitelka, Hansen, and Ashmun 1985). Finally, although many clones have been mapped (Barrett and Thomson 1982, Cook 1985, Maddox et al. 1989, Klimes 1992), there have been no attempts to relate ramet contexts to their age and reproductive success. Although Falinska (1995) presents data for Filipendula ulmaria on the relationship between genet disintegration and both age and co-occurring plant assemblages.

The use of ramet context in this chapter may represent a new way to approach clonal plants. It is not necessary in mayflower, and perhaps other clonal plants as well, to excavate entire clones. Information about relations between ramets can be gathered with minimal excavation.

CHAPTER VI

SYNOPSIS

Populations of Canada mayflower (Maianthemum canadense Desf.), and other clonal organisms are organized at a number of levels. Ramets (the structures that live, reproduce, and die) of a particular genotype are organized into a genet. Populations may be composed of a number of genets. It is possible that many factors may influence the seed maturation of mayflower ramets. These factors may operate at the level of the ramet, the genet, or the population.

The ramets of mayflower differ from one another with respect to the number of seeds that they mature. Ramets differ from one another in other respects as well. Flowering ramets tend to be older than vegetative ramets (Chapter IV and V). The number of years that a ramet waits to flower is variable (Chapter I). Most flowering ramets were flowering for the first time (80.5%), 15.8% were found to be flowering for the second time, and 3.7% were found to be flowering for the third time (Chapter I). Ramets flowering for the second time varied in the number of years that they last flowered and no ramet was found to have flowered in two consecutive years (Chapter I).

Ramets that waited a greater number of years to flower did not necessarily mature more seeds. Ramets flowering for the second time matured as many seeds as ramets flowering for the first time when pollen was not limiting (Chapter III). The addition of pollen did result in an overall increase in the number of seeds matured by ramets (Chapter I, III, and V).

The genets of mayflower may be extensive and diffuse. The rhizomes between ramets may break down over time. This makes the identification of genets by excavation difficult. If the survivorship or reproduction of a flowering ramet is affected by other ramets in the same genet, then more proximate ramets are likely to be involved than are more distant ramets. The context of a ramet may thus be defined as the identity of the ramet directly basipetal and acropetal on the same rhizome system. The frequency of flowering ramets was found to differ among contexts (Chapter IV and V). Flowering ramets with no ramet basipetal or acropetal (isolated ramets) represented the most common context with > 53% of flowering ramets occurring in this context (Chapter IV and V). Isolated flowering ramets were found to be older than non-isolated ramets (Chapter V). In both 1996 and 1997, isolated flowering ramets matured fewer seeds than non-isolated flowering ramets (Chapter IV and V). This trend was also validated experimentally in Chapter I and this experiment clarified the role of pollen in determining the number of seeds matured by ramets. Experimentally isolated ramets that were open pollinated matured as many seeds as non-isolated ramets that were open pollinated. However as the level of pollen increased, non-isolated ramets were able to mature more seeds than experimentally isolated ramets.

Rainfall in June of 1997 was much lower than the rainfall in June of 1994, 1995, and 1996 (Chapter V). It is possible that lower rainfall in June of 1997 limited seed maturation in the ramets of mayflower. Despite the overall reduction in the number of seeds matured in 1997, all flowering ramets were not limited to the same degree. Isolated ramets in 1997 matured fewer seeds than non-isolated ramets (Chapter V) suggesting that isolated ramets were influenced to a greater degree by low rainfall than non-isolated ramets.

It appears that for mayflower, a genet-based approach to explaining variation in the number of seeds matured by ramets is most appropriate. In general the addition of pollen to ramets resulted in an increase in the number of seeds matured; however, the experiment presented in Chapter I demonstrated that not all ramets responded to pollen addition (Ganger 1997). Isolated ramets that received additional pollen did not mature more seeds. Low rainfall in 1997 may have limited the number of seeds matured by ramets overall; however, isolated ramets appeared to have been influenced to a greater degree than non-isolated ramets (Chapter V). The use of a genet-based approach to demography may also be preferable to a ramet-based approach for other clonal species.

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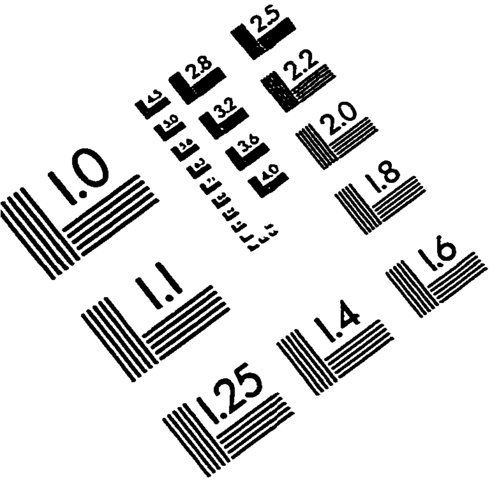
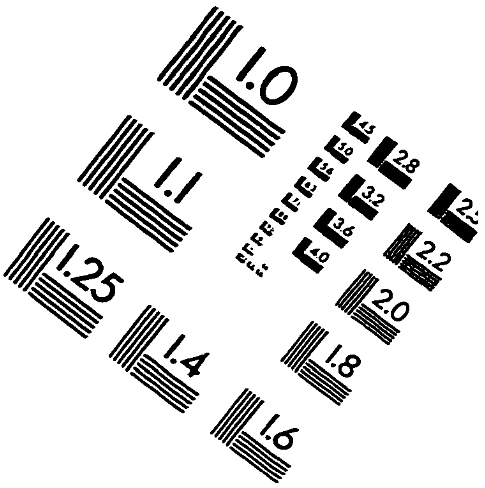
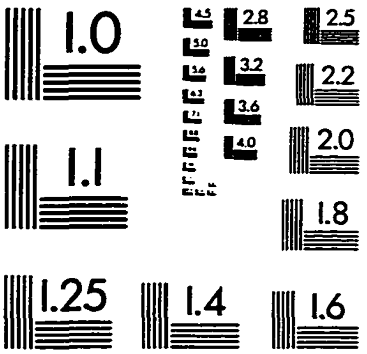
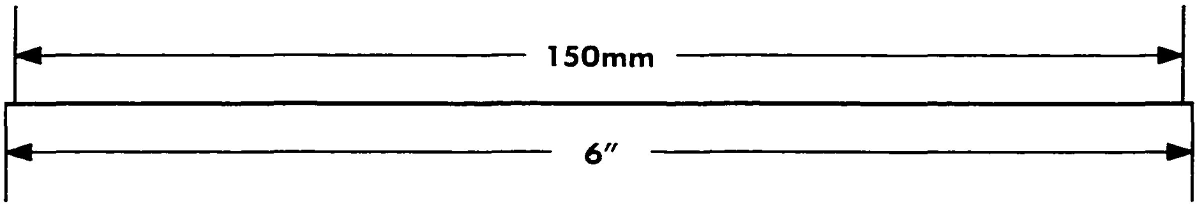
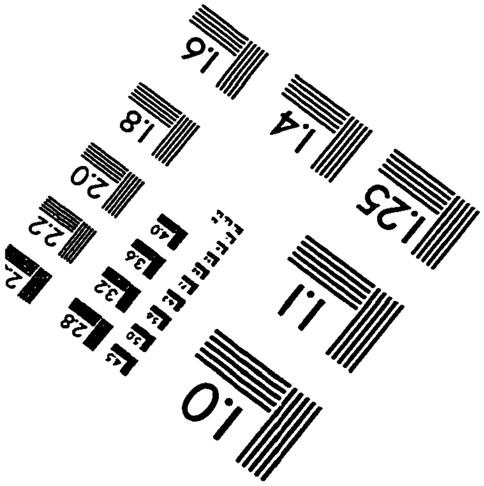
IMAGE EVALUATION TEST TARGET (QA-3)











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