Pollination processes – maternal and offspring performance

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Till Julina och Matilda
Nu ska vi leka igen!

"Knopparna knoppas och sipporna hoppas. Man blir glad av blad."

(Ur Mammu Mu städar av Jujja och Tomas Wieslander, 1997)
Pollination processes – maternal and offspring performance

Abstract – Pollination is one of the most important factors determining the reproductive success of plants. This thesis examines processes associated to varying pollination, with focus on plant responses.

The first aim was to examine the possibility and constraints for short-term evolution of flower size in *Raphanus raphanistrum*. The results showed that there exists a possibility for pollinator-mediated short-term evolution of flower size in the study species. Flower size was strongly correlated to plant size. Since flower size cannot evolve separately from plant size, this correlation may constitute a constraint to the evolution of flower size.

The second aim was to determine how varying pollen load affects later flowering, reproduction and growth of maternal plants. High pollen load treatment resulted in larger or more flowers on late flowers, which may enhance pollen dispersal and reproductive success, while the total seed mass was the same between treatments. The results indicate that the study species *R. raphanistrum, Sinapis arvensis* and *Brassica napus* have plastic responses in floral traits according to the present pollination level.

The third aim was to determine how varying pollen load affects seed quality and offspring vigor. The results suggest that high pollen load had no positive effects on seed quality or offspring vigor due to pollen competition. Instead, seed mass determined seed quality and offspring vigor in the three study species and low pollen load treatment resulted in high-quality offspring due to heavier seeds.

The fourth aim was to examine causes and consequences of variation in reproductive success of *Succisa pratensis* on a regional scale. The results suggest that the most important variables, on a regional scale, for reproductive success were population size and habitat quality. The results showed that seed weight variation might be important when assessing reproductive success. In this study, seed weight variation did not seem to be adaptive.

Keywords: Pollination, *Sinapis arvensis*, *Brassica napus*, *Raphanus raphanistrum*, *Succisa pratensis*, Evolution of flower size, Maternal plant responses, Resource allocation, Reproductive success.
This thesis is comprised of a summary and four papers, which are referred to by their Roman numerals:


IV Holmén Bränn K. and Mildén M. Variation in reproductive success on a regional scale in *Succisa pratensis*. Manuscript.
Introduction

The main ecological mechanisms affecting seed set are pollination success and amount of available resources. Insufficient pollination occurs in most species, at least sometimes, and differences in pollination service may occur at different scales. Plants cope with the variable pollination services in different ways. The overall goal of this thesis is to examine evolutionary and ecological processes, associated to varying pollination services, with focus on plant responses.

Causes of variable pollination services

Pollinator services can vary widely and pollinator behavior and movement is not random. In fact, there are several mechanisms at different levels that may cause variation in pollinator services. At a large scale, the landscape context may affect pollinator diversity and abundance and different pollinator groups may respond differently at different spatial scales (Steffan-Dewenter et al. 2001, Steffan-Dewenter et al. 2002). At the population level there are several factors that may influence pollinator visitation. The population size may affect the number of visiting pollinators. Several studies have shown that small populations suffer from reduced reproductive success due to pollen limitation (Ågren 1996, Hendrix and Kyhl 2000, Kolb 2005, Ward and Johnson 2005). Sparse populations may attract fewer pollinators than dense populations; there may also be an increased risk of receiving more heterospecific pollen in sparse than in dense populations. Local density has been shown to affect pollination and reproductive success and most studies have found a negative relation between density and reproductive success (Kunin 1997, Knight 2003, Metcalfe and Kunin 2006, Wagenius 2006).

The distance to surrounding populations may also affect the possibility for a population to be visited by pollinators. Increasing distance has been shown to cause a reduction in the number of pollinators and increased pollen limitation (Jennersten 1988, Steffan-Dewenter and Tscharntke 1999, Groom 2001). In addition, a study on the annual herb *Clarkia concinna* (Groom 1998) showed that the interaction between population size and isolation affected reproduction due to pollen limitation. Small populations had reduced seed set due to insufficient pollination when a critical threshold of isolation
was exceeded, while large populations attracted a sufficient number of pollinators regardless of isolation.

Among individual plants there may also occur differences in pollination services for several reasons. Pollinators are attracted to flowering traits and several studies have shown that both flower size and the number of flowers affect pollinator visitation (Klinkhamer et al. 1989, Young and Stanton 1990, Conner and Rush 1996, Emms et al. 1997, Vaughton and Ramsey 1998a). In addition, flower color may also affect pollinator visitation (Jones and Reithel 2001). A large plant height has been shown to attract more pollinators resulting in higher pollen deposition (Lortie and Aarssen 1999) and more genetically diverse pollen loads (Carromero and Hamrick 2005). Different pollinators can, however, prefer plants of a certain height (Gumbert and Kunze 1999). In addition to these mechanisms, a variable pollination success can also occur due to variable weather conditions.

**Pollination and reproductive success**

Increased pollinator visitation rates and pollen deposition on stigmas may affect both male and female fitness. Male fitness may be affected since increased pollinator visitation may enhance pollen export and thereby offspring production through male function (Snow and Lewis 1993; Arnold 1994).

Pollen load may affect female fitness through the number of seeds produced. If the number of pollen grains deposited on the stigma limits reproduction, the seed set will increase with additional pollen until all ovules are fertilized. Within a plant, pollen load can also affect the likelihood of fruit maturation and thereby affect seed set. Fruits with many fertilized ovules have been shown to have higher probability to mature than fruits with few fertilized ovules (Winsor et al. 1987, Bertin 1990, Winsor et al. 2000).

Seed quality is often measured by seed weight since it may affect germinability (Tripathi and Kahn 1990, Seiwa 2000), germination time (Maun and Caver 1970, Tripathi and Kahn 1990) and seedling size (Vaughton and Ramsey 1998b, Eriksson 1999). Seed weight is, however, often variable within species (Janzen 1977, Michaels et al. 1988, Winn 1988, Vaughton and Ramsey 1997, Eriksson 1999). This variation may be caused by several factors, for instance by pollination.

In addition to the effect on the number of seeds, many studies have demonstrated increased offspring vigor of seeds from flowers receiving high pollen load (e.g. Mulcahy 1979, Winsor et al. 1987, Bertin 1990, Schlichting et al.
Increased offspring quality may be a result of enhanced maternal investments in flowers with high pollen load (Charlesworth et al. 1987). On the other hand, positive effect on seed quality due to high pollen loads may also occur if a high pollen load results in an increased pollen competition between germinating pollen grains and pollen performance is positively associated with quality at the sporophytic stage. Furthermore, a high number of pollen grains may be associated with a high genetic variation among pollen grains, which may result in increased genetic variation among fertilized ovules and give opportunity for selective seed abortion (Mulcahy 1979). On the other hand, even though a plant receives a large amount of pollen there is also a possibility that the quality of the pollen is poor due to self-incompatibility, inbreeding depression, outbreeding depression, heterospecific pollen and short viability of pollen (Wilcock and Neiland 2002 and references therein).

In contrast to the possibility of positive effects of high pollen loads there is also a possibility that a low pollen load may enhance seed quality. If there are lots of maternal resources available to each fertilized ovule but pollination levels are low, maternal plants may compensate the low offspring number by increasing offspring quality by large seed size (Sakai and Sakai 1995).

Maternal plant responses

It is usually assumed that enhanced reproduction results in reduced growth and future reproduction of a mother plant. Case studies on trade-offs between reproduction and other life-history traits generally support the predictions of the cost of reproduction hypothesis but there are also several studies that have not detected any cost of reproduction (Obeso 2002). Studies that have used supplemental pollination to increase reproduction have found varying responses. Some studies have found a cost of reproduction by varying the level of pollination (Calvo and Horvitz 1990; Primack and Stacy 1998). Other studies have not detected costs when reproduction has been enhanced by supplemental pollination (Calvo 1990; Jennersten 1991). If a high pollen load causes an increase of seed number or seed quality, it leads to an increased investment of resources to seed production. Even though it has been shown that a high investment to seed production may lead to reduction in vegetative growth or later reproduction, there is also a possibility that maternal plants compensate for enhanced reproduction (reviewed in Obeso 2002). If plants are able to compensate for increased investment in reproduction, compensation is probably partial. Some studies have, however, suggested total compensation or even overcompensation after supplemental hand pollination. In Primula veris, supplemental hand pollination of all
flowers in the field increased photosynthetic capacity and enhanced flowering, growth and survival in subsequent years (Lehtilä and Syrjänen 1995). Dudash and Fenster (1997) also found that hand-pollinated individuals had a survival advantage compared to the control group receiving natural pollination levels in *Silene virginica*.

Variable pollination services may also affect evolution of maternal plant traits in form of flowering traits such as flower size. Variation in flower size is common within species (Cresswell 1998; Herrera 2001). This variation may reflect a spatial variation in the availability of pollinators, if short-time pollinator-mediated selection on floral traits exists. In a longer perspective varying selection pressure from different pollinators may be an important mechanism of speciation in plants (Schemske and Bradshaw 1999 and references therein). There is however certain conditions that needs to be fulfilled for short-time evolution of flowering traits to occur. There must be an additive genetic variation for a flowering trait and there must be a covariation between fitness and the flowering trait (Falconer and Mackay 1996). Even if these conditions are fulfilled the evolution of flowering traits may be constrained by trade-offs within the plant.
Aims of the thesis

The causes and consequences of varying interactions between plants and pollinators is a large and complex research area. This thesis deals with a few ecological and evolutionary processes, with focus on plant responses, associated with pollination. Pollinators may exert selection for flower size. For evolution of plant traits to occur there must exist an additive genetic variation for flower size. The first specific aim of this thesis was to determine whether variation of flower size has a genetic component and whether selection on flower size is constrained by trade-offs between flower size and other plant traits. If the level of pollination affects the reproductive output, it may also affect maternal plant traits. The second specific aim of this thesis was to determine how varying pollen load affects later flowering, reproduction and growth of maternal plants. Varying pollen loads may affect seed quality in several ways. The third specific aim of this thesis was to determine how different pollen loads affects seed quality and offspring vigor. On a regional scale there are many factors that may affect reproduction, among them variable pollination levels. The fourth specific aim of this thesis was to examine causes and consequences of variation in reproductive success on a regional scale.
Methods

The studies in this thesis were performed in the greenhouse (paper I and II) and in the common garden (paper I, III and IV) at Stockholm University. In paper IV seed collection and field studies were performed at Nynäs nature reserve in the south east of Sweden, c. 100 km south of Stockholm.

Four different study species were used, *Raphanus raphanistrum* L. (I, II and III), *Sinapis arvensis* L. (II and III), *Brassica napus* L. (II and III) (Brassicaceae) (Figure 1), and *Succisa pratensis* L. (IV) (Dipsacaceae). *Sinapis arvensis*, wild mustard, is self-incompatible and seeds are produced in siliques with 5-12 seeds in each. The seeds used in this study originated from a roadside population in Sollentuna, Sweden. *Raphanus raphanistrum*, wild radish, is also self-incompatible and seeds are produced in siliques with 2-8 seeds in each. The seeds used in this study originated from a roadside population in Lieto, SW Finland. *Brassica napus*, oil seed rape, is a self-compatible agricultural crop and seeds are produced in siliques with 7-19 seeds in each. The seeds used in this study were commercial seeds that originated from a farm in Finland. The main pollinators of these species are honeybees, bumblebees, solitary bees, syrphid flies and butterflies (Conner et al. 1995; K. Lehtilä unpubl. data). *Succisa pratensis* is a long-lived perennial plant mostly found in mesic or moist seminatural grasslands. Flowering time is from August to September. *S. pratensis* flowers with one to six flowerheads each with about 70-110 small violet flowers. The seeds are hidden in a hairy capsule with one seed in each capsule. *S. pratensis* is mainly pollinated by syrphid flies, bees and butterflies.

Selection on flower size

To examine whether variation of flower size has a genetic component and whether selection on flower size is constrained by trade-offs between flower size and other plant traits (paper I) artificial selection for small and large petal size was carried out for two generations. Seeds from 98 maternal plants were used to produce the first generation. Half-sibs were used in generation I and full-sibs in later generations.
In generation 1, the length and width of three petals from three flowers of each individual were measured. Sibling groups of plants with the smallest and the largest average petal size were chosen to produce plants for the next generation. Controlled pollination was used to produce full-sib generation 2 progeny. Generation 1 and 2 were raised in the greenhouse.

In generation 2, the length and width of three petals from three flowers of each individual were measured again and groups of plants with the smallest and the largest average petal size were chosen to produce plants for the next generation.

Generation 3 was raised in the common garden. Again the length and width of three petals from three flowers of each individual were measured. In this generation, start of flowering, individual seed weight, seed number, total seed weight, number of fruits, number of seeds per fruit, stem thickness, plant height, number of branches and flower number was also measured.

We calculated the realized heritability as

\[ h^2 = \frac{R}{S} \]

where \( S \) is the selection differential

\[ S_t = (\bar{z}^{*}_{u,t-1} - \bar{z}_{u,t-1}) - (\bar{z}^{*}_{d,t-1} - \bar{z}_{d,t-1}) \]

and \( R \) is the response to selection

\[ R_t = (\bar{z}_{u,t} - \bar{z}_{u,t-1}) - (\bar{z}_{d,t} - \bar{z}_{d,t-1}) \]

for each generation \( t \). \( \bar{z} \) refers to the family average of petal size, \( \bar{z}^{*} \) refers to petal size of families selected to be parents of the next generation, and \( u \) and \( d \) refer to upwardly and downwardly selected lines, respectively (Walsh 1999).

Narrow-sense heritabilities and genetic correlations of plant traits were estimated by using a restricted maximum likelihood (REML) approach with the VCE 5.1.2 package (Neumaier and Groeneveld 1998; http://vce.tzv.fal.de/index.pl/vce).
Pollination load effects on maternal plant performance and reproductive success

A hand pollination experiment and a germination experiment were conducted to study the effects of different pollen loads on maternal plant performance, seed quality and offspring quality (paper II and III). The experiments were conducted in a greenhouse and in a common garden.

Fifty pairs of half-sibs (S. arvensis and R. raphanistrum) or 50 pairs of plants with the same starting date of flowering (B. napus) were used. One plant of each pair was randomly assigned to the low pollen load treatment and the other one to the high pollen load treatment. One individual of each half-sib pair received a low and the other one a high pollen load on the 30 earliest flowers. Pollen was collected from 20 anthers from ten individuals that were unrelated to the focal plant. The tip of a nylon line was used to apply pollen to the stigmatic surface. We used a nylon line of 0.2 mm diameter for low pollen load (188 ± 21.6 pollen grains) and a line of 1 mm diameter for high pollen load (697 ± 48.7 pollen grains). Later flowers received high pollen load by pollination with a brush in both experimental groups.

Maternal plant responses were measured by flower size on one flower on later flowers, number of flowers, thickness of the stem at the base of the plant and plant height. The reproductive output was measured by the number of seeds, number of fruit with seeds and seed weight.

Seed quality and offspring vigor was measured in a germination experiment in the common garden. Seeds from flowers treated with high or low pollen load were sown individually in small pots. Germination was followed daily and the size of the cotyledonous leaves was measured 20 days after germination. For S. arvensis the date of flowering, height at flowering start and the final height was also measured.
Causes and consequences of variation in reproductive success on a regional scale

To examine causes and consequences of variation in reproductive success on a regional scale, seeds of *Succisa pratensis* were collected from 52 populations. The effects of population size, isolation, habitat type and management on seed set and seed quality were examined (paper IV). Seeds were counted and the number of viable, aborted and preyed seeds was recorded. From each individual, 8-15 seeds were individually weighed and variation in seed weight was quantified by using the coefficient of variation.

As a measure of population size we used the number of flowering individuals. Population isolation was measured as

\[ S_i = \sum \exp(-\alpha d_{ij}) N_j \]

where \( N \) is the population size (the number of flowering individuals), \( d \) is the distance between patches (between the central points of each patch) and \( \alpha \) is a constant describing how fast the number of migrants from patch \( j \) declines.
with increasing distance (Hanski 1999). Management was categorized as “grazed” and “not grazed”. For classification of habitat type we recorded whether the populations were situated in grassland or forest and whether the populations were situated in a dry, mesic or moist habitat.

Seed quality was studied in two experiments, one in the greenhouse and one in the field. In the greenhouse, germination and seedling development were followed during two months. In the field, germination and establishment were checked one and three years after sowing.

To examine if decreased seed set in small populations of *S. pratensis* was caused by pollen limitation an additional pollination study was performed in ten of the study populations in the year 2002 (Holmén Bränn unpublished). The pollination experiment was carried out in ten of the populations used for seed collection in paper IV. The number of flowering individuals was used as a measure of population size. Population size ranged from 100 to 1000 flowering individuals. In each population five individuals was used as control. Two pollination treatments were used: extra pollen from the own population, extra pollen from a large population about 100 m away. The pollination was made with a brush. Eight individuals per population were used in each treatment and each individual was pollinated at three separate occasions. Seed were collected after maturing. Some of the pollinated plants were destroyed by grazing, trampling and seed predation resulting in that 85 pollen treated plants and 50 control plants were used in the analyses. In each population the number of pollinators was recorded in a square of 0.5*0.5 m for twenty minutes at each pollination occasion in the population. The tree pollinator observations were made at different times during the day and the weather conditions were noticed. The seeds were counted and 15 seeds from each individual were weighed with their capsules. Differences in seed quality due to different pollination treatments were examined in a field experiment. Seeds were sown in a mesic meadow in Nynäsh nature reserv. Seven 10×10 cm squares per population and treatment were used. Twenty seeds were sown in each square. In the field, germination and establishment were checked one and three years after sowing.
Results and Discussion

Correlated effects of selection for flower size

Pollinators may exert varying selection pressures on flowering traits and one of these traits is flower size. For evolution of flower size to occur, the first condition to be fulfilled is an additive genetic variation for flower size. In paper I the potential for short time evolution of flower size was examined in R. raphanistrum. Artificial selection on flower size showed a realized heritability $h^2=0.49$, a result in line with other studies of flower size selection (Worley and Barrett 2000, Lendvai and Levin 2003, Delph et al. 2004). The second condition to be fulfilled is a positive covariation between fitness and flower size. This covariation was not examined in this study, but other studies on R. raphanistrum have shown that large flowers attract more pollinators (Conner and Rush 1996) and that flower size is positively correlated with seed production (Conner et al. 1996a). Since large flowers attract more pollinators this may also increase pollen export and thereby male fitness (Young and Stanton 1990, Ishii and Sakai 2001, Ehlers et al. 2002). Thus, the study species fulfill the conditions for short-term evolution of flower size to occur. Even though it is likely that there is a positive correlation between flower size and fitness according to the interaction with pollinators, there is also a possibility that herbivores or pre-dispersal seed predators are attracted to large flowers (Galen 1999). In that case, there is a possibility that pollinators and seed predators exert antagonistic selection pressures on flower size.

An increased flower size may demand a lot of resources and bring down costs upon other plant traits, which may constrain the evolution of flower size. Potential costs of producing large flowers may be expressed through reduced number of flowers, reduced reproductive output or reduced vegetative growth. Constraints due to such trade-offs with other plant traits were examined in paper I. Several reproductive and vegetative traits were measured. Only a few trade-offs between flower size and other plant traits were found. The genotypic lines selected for large flowers started to flower later than the lines with small flowers. The lines with large flowers also produced significantly lighter seeds and had a lower number of seeds per fruit than the lines selected for small flowers. There were, however, no differences in the
total seed mass, indicating that both lines had the same reproductive output. Instead of a trade-off between flower size and other traits, the results in paper I showed several positive correlations between flower size and other plant traits. The genotypic lines selected for large flowers produced significantly more flowers than the genotypic lines selected for small flowers. The genotypic lines with large flowers also had a larger final height, thicker stem and more branches than the lines with small flowers. In addition, the genotypic correlation between flower size and final height was positive. Thus, the results indicate that flower size is strongly correlated with plant size and thereby flower size cannot evolve separately from plant size. A positive correlation between flower size and other plant traits may, however, instead constrain the evolution of flower size if there are contrasting selection pressures on these traits (Worley and Barrett 2001). The differences between selection lines in flowering start that was found in paper I suggest that genotypes with large flowers and larger plant size spend more time to accumulate more resources before flowering than genotypes with small flowers. Patterns of negative genetic correlation between age and size at first reproduction have been found within and among several wild populations of Brassica rapa (Mitchell-Olds 1996). Since it can be favorable to have an early flowering start (Widén 1991), a trade-off between age and size at flowering start can be important in the evolution of flower size.

### Pollen load effects on maternal plant performance and reproductive success

Varying pollinator activity may alter the pollen load received by a plant. A high pollen load may enhance reproduction and it is usually assumed that enhanced reproduction results in reduced growth and future reproduction of the mother plant (Obeso 2002). There is, however, also a possibility that maternal plants compensate for enhanced reproduction or that a high pollen load increases resource intake (Obeso 2002). Paper II examined how varying pollen load affects later flowering, reproduction and growth of maternal plants. In S. arvensis high pollen load treatment resulted in larger flowers in late flowering compared to low pollen load treatment. The high pollen load resulted in more but lighter seeds in early flowers while in the whole plant level there were no differences in seed number or total seed mass between the treatments. In addition the high pollen load treatment resulted in fewer fruits. Thus, the reason for larger flowers in the high pollen load treatment was probably that plant receiving a low pollen load treatment had to spend more resources on maximizing the number of seeds than the plants treated with the high pollen load treatment and thereby plants receiving a low pollen load had a reduced amount of resources available for floral traits. In R. rap-
high pollen load treatment resulted in more flowers than low pollen load treatment. The plants that received high pollen load produced fewer fruits but there were no differences total seed mass between the treatments at any level. Thus, low pollen load treatment resulted in more resources spent on fruit production than in the high pollen load treatment. Thereby low pollen treated plants had a reduced amount of resources available for floral traits, which probably is the reason for the reduced flower number in the low pollen load treatment in *R. raphanistrum*. In *B. napus*, there were no differences between the treatments in flowering traits or reproductive traits. There were no effects on the vegetative traits between treatments in any of the species. In summary, both pollen load treatments resulted in the same female reproductive output and, in addition, high pollen load had positive effects on floral traits on late flowers that may enhance pollen dispersal. Thus, the results suggest that plants receiving a high pollen load may have an enhanced reproductive success when both seed production and pollen dispersal are taken into account. Positive effects on flowering traits due to high pollen loads have been found in a field study on *Primula veris* (Lehtilä and Syrjänen 1995). In their study supplemental hand pollination of all flowers increased photosynthetic capacity and enhanced flowering, growth and survival in subsequent years (Lehtilä and Syrjänen 1995). However, the most probable explanation for the positive responses on flowering traits due to high pollen loads found in paper II was resource allocation between early and late flowering.

Offspring vigor has been shown to increase on seeds from flowers receiving high pollen load (e.g. Mulcahy 1979, Winsor et al. 1987, Bertin 1990, Schlichting et al. 1990). Enhanced maternal investments, enhanced pollen competition between germinating pollen grains or enhanced genetic variation among fertilized ovules may cause improved seed quality. In paper III the results showed that seeds and seedlings from plants receiving a high pollen load treatment did not have higher quality compared to seeds from low pollen treatments. In *R. raphanistrum* and *B. napus*, where there were no differences in number of seeds and mean seed weight between treatments (paper II), high pollen load treatment did not increase germination probability, offspring size or survival. In *R. raphanistrum* seedling survival and cotyledon size were positively affected by seed weight. In *B. napus*, cotyledon size also was positively affected by seed weight while positive effect of seed weight on seedling survival was only marginally significant. The results suggest that seed weight, irrespective of the size of the pollen load, determines seed quality in these two species. Thus there were no indications of enhanced seed quality due to pollen competition in these two species. Several studies have indeed found a positive effect of high pollen load on offspring vigor (e.g. Mulcahy 1979, Winsor et al. 1987, Bertin 1990, Richardson and Stephenson 1992, Palmer and Zimmerman 1994, Niesenbaum 1999
and references therein) while several studies have not found any positive effect of high pollen load size (Smith et al. 1990, Snow 1990, 1991, Mitchell 1997, Marshall et al. 2000). There are some possible explanations for varying results on seed quality, such as growing conditions (Kalla and Ashman 2002), variation among taxa or the possibility that composition of pollen donors is more important than the size of the pollen load (Marshall et al. 2000). In addition, Mitchell (1997) suggested that the level of pollen load sizes that is used in the small pollen load treatment often is too high to find any effects of pollen competition since pollen competition then occurs in both treatments. The pollen load treatments used in paper III represented pollen load levels that in the low pollen load treatment were nearly sufficient for a full seed set and the high pollen load treatment a level of ample pollen load. The low pollen load was thereby quite high and this may contribute to the absence of a positive effect of high pollen load.

If pollen competition does not enhance seed quality, the size of the pollen load may affect seed quality by affecting the amount of resources available for each seed. A low pollen load may result in a decreased seed number and thereby more resources may be available to each maturing seed. Plants receiving a low pollen load may thereby produce few but heavy seeds of high quality. In *S. arvensis*, low pollen load treatment resulted in the same total seed mass as high pollen load treatment, but low pollen treatment resulted in fewer and heavier seeds than high pollen load treatment (paper II). In paper III, the seeds from low pollen load treatment had higher germination probability, larger cotyledon size and higher survival to flowering when seed mass was not controlled for by covariance analysis. When seed mass was controlled for, pollen load did not affect cotyledon size or survival to flowering. Thus, there was a positive effect of low pollen load treatment due to larger seeds in *S. arvensis*. In addition, the probability to germinate was positively affected by low pollen load even when controlling for seed mass. Cotyledon size was positively affected by seed weight in *S. arvensis*. As in the other two study species, there were no positive effects of a high pollen load in this species. The results, thus, suggest that seed mass in a large part determines seed quality. However, the size of the pollen load can affect seed quality by affecting seed mass. When seed number was low due to a low pollen load seed quality was enhanced through higher seed mass.

In *S. arvensis*, development after the cotyledonous stage was followed to examine whether seed mass and pollination treatment affected later development. Seed mass, but not pollination treatment, was positively associated with early flowering start, the height at flowering start and final height. Tall plants have been shown to attract more pollinators, probably because they are more visible to pollinators, resulting in higher pollen deposition (Lortie and Aarssen 1999) and more genetically diverse pollen loads (Carromero
and Hamrick 2005). Early flowering start has also been shown to be positively associated to seed production (Widén 1991). These results suggest that seed weight irrespective of pollen load treatment also affects plant development and plant fitness after the seedling stage.

Causes and consequences of variation in reproductive success on a regional scale

There are several factors, acting on different spatial scales, which can affect seed set. On a regional scale, population size and isolation may both influence seed set. Small populations may suffer from reduced seed set due to pollen limitation (Ågren 1996, Hendrix and Kyhl 2000, Kolb 2005, Ward and Johnson 2005). The connectivity with surrounding populations may be associated with pollen limitation (Jennersten 1988, Steffan-Dewenter and Tscharntke 1999, Groom 2001). Small populations may suffer from pollen limitation both due to a reduced number of pollinators, greater relatedness and incompatibility among individuals. There are also other genetical effects that may reduce fitness in small and isolated populations, such as founder effects, genetic drift and reduced inter-population gene flow (Young et al. 1999). There is also a possibility that positive relationships between population size and fitness may arise due to differences in habitat quality. In paper IV the causes and consequences of variation in reproductive success on a regional scale was examined. The results showed that seed set was positively associated to population size but not to connectivity, habitat type or management. Mean seed weight was not associated to population size or connectivity. Mean seed weight was higher in ungrazed than in grazed populations. Seed weight variation was substantial and associated to the proportion of ovules setting seeds but not to population size, connectivity, habitat type or management.

Reduced seed set in small populations may occur due to pollen limitation, poor environmental conditions or genetical effects. Since small populations suffered from reduced seed set independent of connectivity, habitat type or management, the most likely explanations for decreased seed set in small populations of *S. pratensis* are pollen limitation or genetical processes. Connectivity did not have any effects on seed number, mean seed weight or seed weight variation in our populations of *S. pratensis*. The study in paper IV was made in a landscape where semi-natural grassland habitats are declining, although there still exist intact habitats for several populations. There is a possibility that the distance and size of the populations in this area has not yet reached the critical level for connectivity to matter for seed set. Another possibility is that the measure of connectivity used in this study does not
represent the correct connectivity measure for all processes that can affect seed set, even though several measures were tested.

To examine whether decreased seed set in small populations of *S. pratensis* was caused by pollen limitation, a pollination study was performed in ten of the study populations in the year 2002 (Holmén Bränn unpublished). The results from the pollination study contrasted to the prediction in paper IV. There were no effects of pollination treatment on mean seed weight, mean number of viable seeds or mean number of aborted seeds when using population size as a covariate (Holmén Bränn unpublished). However, there was no association between population size and seed set in this year (Holmén Bränn unpublished), which can explain the lack of response to pollination treatment. In addition, there was no association between the number of pollinators per plant and population size (Holmén Bränn unpublished), which also indicates that there was no difference in pollinator services due to population size. Probably reduced reproductive success caused by pollen limitation differs between years. Varying pollen limitation between years has been found also in other species (Burd 1994; Ågren 1996, Baker et al. 2000). There were no significant differences between treatments with pollen from the own population and pollen from a population 100 m away, according to seed weight, number of viable seeds or number of aborted seeds (Holmén Bränn unpublished). These results indicate that there was no reduced interpopulation pollination or inbreeding in the study populations. In addition, association between population size and seed set differed between years, which may indicate that there were no other genetical effects such as founder effects or genetic drift in small populations. Pollen limitation may differ between years but founder effects or genetic drift should probably be expressed in both years.

Plant population size and connectivity have also been shown to affect the abundance of pre-dispersal seed predators (Jennersten and Nilsson 1993, Ehlers and Olesen 2003, Elzinga et al. 2005). In this study the results showed that pre-dispersal seed predation was positively associated to population size but there was no association with connectivity. However, pre-dispersal seed predation did not seem to have any large effects on seed production in the study populations.

Seed quality is often estimated by seed weight. In this study seed quality was estimated by germination experiments, taking seed weight, seed weight variation, population size, population connectivity, management and habitat into account. Seed weight variation was studied since large variation in seed mass within species is common in several species (Janzen 1977, Michaels et al. 1988, Winn 1988, Vaughton and Ramsey 1997, Eriksson 1999) but little is known about its actual fitness effects. In the greenhouse, seed weight was
positively associated to cotyledon size and negatively associated to germination time. There was no association between mean seed weight and germination. Seed weight variation had a positive effect on cotyledon size but there was no effect of seed weight variation on germination or time to germination. Seed quality was affected by grazing and moisture gradient but not by population size or connectivity. In the field experiment, mean seed weight was not associated with germination or establishment. Seed weight variation was neither associated with germination but there was a tendency that mean individual coefficient of variation was negatively associated with the number of established seedlings. Pollination treatment with pollen from the own population and pollen from a population 100m away did not have any effect on germination and establishment (Holmén Bränn unpublished).

Few empirical studies have examined the effect of seed weight variation on recruitment. The results in paper IV showed that seed weight variation affects establishment but not germination. The effects were, however, contradictory. In the greenhouse, seed weight variation had a positive effect on cotyledon size while in the field, seed weight variation tended to have a negative effect on establishment. In the greenhouse the environment for the seeds is probably more favorable compared to field conditions, where the smallest seeds may not be able to establish at all. Thus, the most reasonable conclusion is that a large seed weight variation had a negative effect on establishment in the field. In addition, high seed weight variation seems to be related to a poor seed set. Thus, it does not seem likely that seed weight variation is an adaptive trait in \textit{S. pratensis}. However, it is worth pointing out that there may exist certain situations were a variable seed weight might be favorable. For example, seed weight variation might be favorable if dispersal is favored by variation in seed weight or seed predation is associated to seed weight (Geritz 1995, Geritz 1998).
Pollination services have natural fluctuations caused by several factors at different scales. In addition, the interactions between plants and pollinators may also be affected by environmental changes and changes in the landscape structure. This thesis deals with processes, both evolutionary and ecological, associated to varying pollination services with focus on plant responses.

Paper I deals with the possibility for short-term evolution of flower size. The results showed that there exists a possibility of short-term evolution of flower size in the study species *R. raphanistrum*. Pollinators may select for a large flower size since there may be a positive covariation between fitness and flower size due to pollination. However, there is also a possibility of contrasting selection pressures from seed predators and herbivores, resulting in a negative covariation between fitness and flower size. The results also showed that flower size was positively correlated to plant size. Since flower size cannot evolve separately from plant size, the correlation between flower size and plant size may constitute a constraint to the evolution of flower size if there are contrasting selection pressures on these plant traits. In this study we found one example of one such possible constraint, since flowering start was associated to flower size and plant size.

Paper II and III examined responses of different pollen loads on vegetative traits, flowering traits, seed production, seed quality and offspring vigor. The results suggest that plants receiving a high pollen load may have an enhanced reproductive success, taking both seed production and pollen dispersal into account, due to larger or more flowers high up in the inflorescence. However, according to seed quality and offspring vigor, high pollen load treatment did not increase germination probability, offspring size or survival. Thus, there were no indications of enhanced seed quality or offspring vigor due to pollen competition in this study. The results suggest that seed weight, irrespective of the size of the pollen load, determines seed quality in the study species. The size of the pollen load can, however, affect seed quality by affecting seed weight. The low pollen treatment may lead to fewer but heavier seeds and thereby reduced fitness due to reduced number of seeds can be compensated with high-quality seeds. The results also indicate that the study species were able to adjust resource allocation to optimize fitness
according to the present pollination level. Plastic traits may be favorable under variable environmental conditions and there is a general agreement that adaptive plasticity can evolve under natural selection (Schlichting 1986). Sultan (2000) concluded that plastic switches can occur in response to the internal environment of the plant, resource availability or other external cues. There are some studies that have found plastic responses in reproductive traits due to variable environmental conditions (Diggle 1994, Galloway 1995). Pollination may also constitute an external factor that causes plastic responses. Plastic responses have been found in the degree of self-incompatibility due to variable pollination conditions (Vogler et al. 1998) and floral display size (Harder and Johnston 2005). Pollination is indeed variable and thereby plastic responses due to variable pollination levels may be adaptive.

Paper IV examined causes and consequences of variable reproductive success on a regional scale. The results suggest that when assessing variable reproductive success on a regional scale, both population size and habitat quality are important to be taken into account. Population size was the most important factor affecting seed set in the study populations while management regime and moisture gradient affected seed quality. Pollen limitation was the most probable cause of reduced seed set in small populations but pollen limitation varied between years, showing that causes of variable reproductive success on a regional scale may differ between years. The results also showed the importance of assessing seed quality by germination experiments. In addition to mean seed weight, population size, connectivity, habitat and management, the effects of seed weight variation on reproductive success was examined. The results suggest that seed weight variation is important to take into account when assessing seed quality. Large seed weight variation was associated to reduced seed set and tended to have negative effects on establishment. Thus, seed weight variation did not seem to be adaptive in *S. pratensis.*
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Svensk sammanfattning

Växter står still och därmed är interaktionen med pollinatörer som transporterar pollen mellan individer och populationer mycket viktig för växternas reproduktion. Antalet pollinatörer som besöker en växt kan variera beroende på många olika faktorer så som väder, landskapets struktur, storleken och strukturen på populationen individen ingår i, avståndet till andra populationer, storleken på individen, antalet blommor på en individ och storleken på blommorna.


På grund av att pollinatörer har en stor inverkan på reproduktionen påverkas också moderplantan på olika sätt. En ökad reproduction kan leda till att andra delar av växten får en reducerad mängd resurser men det förkommer också att moderplantan kan kompensera för den ökade reproduktionen. Det finns också några få exempel på att växter kan anpassa blomningen efter rådande pollinations förhållanden. Interaktioner mellan växter och pollinatörer kan påverka evolutionen av olika blomkaraktärer, där de karaktärer som leder till en stor reproduktiv framgång för att de lockar många pollinatörer gynnas. I ett längre perspektiv kan också nya arter utvecklas genom en sådan pollinatörssdriven evolution av blomkaraktärer.

Det övergripande målet med denna avhandling var att undersöka ekologiska och evolutionära processer associerade till varierande pollination, med utgångspunkt från växtens perspektiv.

Om en pollinatörssdriven evolution av blomstorleken ska vara möjlig måste blomstorleken vara ärfigt. Evolutionen av blomstorleken kan begränsas om stora blommor kräver mycket resurser som påverkar andra delar av individen negativt. Den första studien i avhandlingen under söker om blomstorleken hos Åkerrättika är ärfigt samt om en potentiell utveckling av blomstorleken är begränsad av en resursavvägning mellan olika växtdelar.
Ett selektionsförsök i två generationer på blomstorlek genomfördes på Åkerrättika (Raphanus raphanistrum) och försöket utfördes i växthuset på Stockholms Universitet.

Hur mycket pollen en individ får kan påverka reproduktionen och därmed också själva moderplantan. Den andra studien i avhandlingen undersöker hur olika pollenmängder påverkar hela moderplantan det vill säga reproduktion, vegetativ tillväxt och blomkaraktärer. Pollinationsförsöken utfördes i växthuset på Stockholms Universitet. Två olika pollenbehandlingar användes och som studiearter användes Åkerrättika (Raphanus raphanistrum), Åkersenap (Sinapis arvensis), Raps (Brassica napus).

Pollen mängden påverkar hur många fröer som produceras men den kan också påverka också frökvaliteten. Den tredje studien i avhandlingen undersöker hur olika pollenmängder påverkar hela moderplantan det vill säga reproduktion, vegetativ tillväxt och blomkaraktärer. Pollinationsförsöken utfördes i växthuset på Stockholms Universitet. Två olika pollenbehandlingar användes och som studiearter användes Åkerrättika (Raphanus raphanistrum), Åkersenap (Sinapis arvensis), Raps (Brassica napus).

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avvägning mellan blomstorlek och blomningsstart, individer med stora blommor började blomma senare. Eftersom det kan vara positivt att börja blomma tidigt kan detta vara ett exempel på en begränsning i evolutionen av blomstörleken.


Resultaten i den tredje studien visade att frön från individer behandlade med den höga pollen mängden inte hade bättre kvalitet än de från individer behandlade med den lägre pollen mängden. Åkerrättika och Raps hade samma antal och medelfrövikt i de olika behandlingarna men pollen behandlingen gav ingen effekt på groning, groddplantsstorlek eller överlevnad. Däremot hade fröstorleken en positiv effekt på groddplantsstorlek och överlevnad hos Åkerrättika och på groddplantsstorlek hos Raps. Att det inte blev några positiva effekter kan bero på att pollenkonkurrens inte påverkar frökvaliteten i dessa arter men det kan också bero på att båda pollenmängderna var höga och att pollenkonkurrens förkommer i båda behandlingarna. Om pollen konkurrens inte påverkar frökvaliteten så kan pollenmängden påverka frökvaliteten genom andelen resurser som finns tillgängliga för varje frö. En låg pollenmängd kan då resultera i att färre men större fröer. De individer av Åkersenap som fick den lägre pollenmängden producerade färre men större frön i början av fröproduktionen och dessa fröer hade en högre grobarhet, större groddplantor och bättre överlevnad. Dessa resultat visar att en lägre pollenmängd kan resultera i fröer av bättre kvalitet på grund av en ökad resurstillgång. Hos åkersenap följes utvecklingen av plantorna genom hela blomningssäsongen. Fröstorleken var positivt korrelerad med blomningstart, höjden vid första blomning och slut höjden. En tidig blomning kan vara
positiv för fröproduktionen och lika så höjden. Resultaten visar att fröstorleken kan ha en positiv inverkan i alla stador av individens liv.


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