

A vertical stem of a terrestrial orchid, likely a species of *Isotria medeolae*, is shown against a dark green background. The stem is green and bears several small, light-colored flowers with yellow centers and long, slender petals. The flowers are arranged in a loose, vertical cluster along the stem.

Elisa Mattila

Factors Limiting Reproductive
Success in Terrestrial Orchids

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Factors Limiting Reproductive Success in Terrestrial Orchids

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Success in Terrestrial Orchids



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ABSTRACT

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Factors limiting reproductive success in terrestrial orchids

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Resource and pollen availability are generally regarded as the most important factors limiting reproductive success of plants. Pollination limitation has been shown to play an especially important role in orchids with complicated pollination systems. However, resource availability may also affect reproductive success in perennial plants both directly and indirectly via pollinator attraction. In this thesis, I examine the importance of both pollen and resource availability to reproductive success in nectarless and nectar-producing orchid species. I also examine how the position within an inflorescence affects reproductive traits of individual flowers. Reproductive success of nectarless *Dactylorhiza incarnata* was found to be primarily pollination-limited within a year, while in nectar-producing *Platanthera bifolia*, reproductive success was limited by both pollinator services and resources. A decrease in photosynthetic area following artificial defoliation resulted in decreased seed production, but had no effect on pollen length or nectar production in *P. bifolia*, but the defoliated plants were found to have lower pollination success than the non-defoliated plants. In *D. maculata*, inhibition of photosynthesis in both leaves and the photosynthetically active green parts of the inflorescence resulted in decreased seed production. Likewise, inhibition of function of mycorrhizal fungi affected seed production negatively. The underground corm was found to be an important source of resources in this species. In *D. maculata* flower size, pollen production and capsule weight decreased towards the top of inflorescence. Decrease in flower size and pollen production may be caused by architectural effects, but seed production of the uppermost flowers was found to be limited by resource availability. Changes in resource availability and allocation to capsule production did not affect growth and reproduction of *P. bifolia* in the following year. However, in *D. incarnata* heavy allocation to reproduction in one year resulted in decreased probability of flowering in the following year. In *D. maculata*, defoliation decreased leaf area and the amount of resources stored in the underground corm. This difference in life-history traits between rewarding and deceptive species may be due to uncertainty of pollination in the nectarless species.

Key words: *Dactylorhiza*; defoliation; flower position; mycorrhiza; nutrients; *Platanthera*; pollen production; pollinator attraction; seed production; water stress.

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LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following articles, which will be referred to by their Roman numerals. I have planned, performed and am the main writer of papers I, IV, and V.

- I Mattila, E. & Kuitunen M. 2000. Nutrient versus pollination limitation in *Platanthera bifolia* and *Dactylorhiza incarnata* (Orchidaceae). - Oikos (in press)
- II Mattila, E. 2000. The effects of water stress and pollen availability on reproductive success of *Platanthera bifolia* (Orchidaceae). – Det Norske Videnskaps-Akademi. I. Matematisk-Naturvidenskapelige Klasse. Avhandling. Ny serie. (in press)
- III Mattila, E. 2000. Sources of resources for seed production in *Dactylorhiza maculata* (Orchidaceae). (Submitted)
- IV Mattila, E. & Salonen, V. 2000. Effects of defoliation on male and female reproductive traits of a perennial orchid, *Dactylorhiza maculata*. (Submitted)
- V Mattila, E & Salonen, V. 2000. The effect of defoliation on attractiveness to pollinators and reproductive success in *Platanthera bifolia* (Orchidaceae). (Submitted).
- VI Mattila, E. 2000. Position-dependent reproductive success of flowers in *Dactylorhiza maculata* (Orchidaceae). (Submitted)

1 INTRODUCTION

1.1 General introduction

Resource and pollen availability are generally the most important factors limiting reproductive success of flowering plants. Both of these factors have also been found to play an important role in the reproduction of orchids (Ackerman & Montalvo 1990). Almost all studies assessing reproductive success in orchids have concentrated on female traits (usually fruit/flower -ratios). Many of these studies have concluded that orchid species are pollination-limited within a season (Nilsson 1992) reflected as low capsule production and the deceptive pollination strategy of many species. However, only few species can be regarded as pollination-limited over lifetime and studies with terrestrial orchid species have suggested also acute effects of resource availability on reproductive success of these perennial plants (e.g. Whigham 1984). Recent studies with other plant families have also shown more complicated relationships between resource availability and pollination success (e.g. Lehtilä & Strauss 1999).

Terrestrial orchids are usually very long-lived polycarpic species that store resources in an underground corm or in rhizomes. Many species (e.g. *Dactylorhiza* spp.) offer no nectar for pollinators, and pollination is based on either mimic or non-mimic food deception of naive pollinators or pseudocopulation (Nilsson 1992). Nectar-producing orchids usually have well-developed pollination systems (long spurs, toxic nectar etc.). In the majority of European orchid species pollen occur in tetrads that are aggregated into two sectile pollinia. These pollinia form pollen dispersal units that are removed when an appropriate insect visitor comes into contact with the adhesive viscidium at their base (Neiland & Wilcock 1995). One pollinium has been thought to be sufficient for pollinating a number of different flowers, but recent observations in Europe have found insufficiency of pollen deposition in some species (e.g. Lammi 1999).

1.2 Factors limiting reproduction in orchids

1.2.1 Pollen availability

Reproductive success of plants may be limited by number of factors. Insufficient pollen available to stigmas may decrease seed set especially in species with complicated reproductive systems (Ayre & Whelan 1989). Orchid species in general are regarded as pollination limited (Nilsson 1992). The visitation rate of pollinators has been shown to be infrequent and to limit the number of fruits, especially in deceptive orchid species (Fritz & Nilsson 1994, Neiland & Wilcock 1998), but also in rewarding species (Inoue 1985). Almost all European orchid species have pollinia composed of massulae held together by elastoviscin (Pacini & Franchi 1996). Pollen loads found on stigmas are on average only one quarter of the number of massulae in one pollinium (Neiland & Wilcock 1995). Seed production may thus be limited by insufficient pollination even after a pollinator visit (Mehrhoff 1983).

In addition to plant size, which is strongly associated with the amount of stored resources, pollinator attraction may also be limited by current resource availability. Increasing allocation to pollinator attraction and rewards may increase pollination success (Stanton & Preston 1988). Pollinator visitation may rate correlate positively with both nectar production (Real & Rathcke 1991) and spur length (Inoue 1986). Limited resource availability e.g. as a result of defoliation by herbivores may decrease attractiveness to pollinators via a decrease in number of flowers, in corolla size (e.g. Lehtilä & Strauss 1999) or in nectar production (Strauss et al. 1996).

Pollinators usually favour large inflorescences because of the expected positive correlation between display size and reward (Cohen & Shmida 1993). Pollinators have been found to prefer large inflorescences in the terrestrial orchids, e.g. in *Orchis spitzelii* (Fritz 1990). However, a study on the nectarless *Dactylorhiza incarnata* revealed that the effect of inflorescence size on reproductive success varied between different populations probably due to variation in availability of naive pollinators and irregular pollinator movements (Lammi 1999). In plants with sequentially opening flowers a large inflorescence also means longer flowering time (Cole & Firmage 1984). In deceptive species plants with a large inflorescence may, however, receive relatively less pollinations than those with a smaller inflorescence, because the pollinators learn to avoid nectarless flowers (Montalvo & Ackerman 1987).

1.2.2 Nutrient and water availability

The effect of nutrient availability on fruit and seed production of plants has been assessed in numerous studies (see Jeffrey 1987 for review). Nutrient application has been found to increase both growth and seed production in perennials either within a season (Stephenson 1984) or in subsequent seasons (e.g. Shaver & Chapin 1995). However, nutrient application has been reproted

to affect orchid performance negatively (e.g. Dijk & Eck 1995, McKendrick 1996). Studies with terrestrial orchids have mostly examined the effects of nutrient application on growth of juvenile phase and the negative effects found have been due to toxicity of ammonium ions (e.g. Dijk & Eck 1995). Decreased reproductive success and survival following fertilisation in adult orchids may be caused by interference of symbiotic interactions between the orchid and its mycorrhizal fungi (McKendrick 1996) or intense competition for nutrients with other plants (Shaver & Chapin 1995). However, in orchids that the need of macronutrients may exceed the available amount of resources stored in the underground corm and that plants have to assimilate nutrients from the soil at the time of growth and reproduction (Whigham 1984). Besides nutrient stress, insufficient water availability may decrease growth and reproductive success of plants (e.g. Black 1968). The yield components most affected by drought are the fruit number and seed weight (Black 1968). This decrease in female reproductive success due to poor water conditions may be due to decreased nutrient uptake (Etherington 1975). Resource availability has been found to affect also male traits, i.e. quantity and quality of pollen produced (e.g. Aizen & Raffaele 1996), but there are no studies assessing the effect of drought on male traits.

1.2.3 Availability of photosynthates

A decrease in photosynthetic area following defoliation has been found to decrease seed production either due to decreased flower production (e.g. Stephenson 1984) or increased abortion of fruits and seeds (e.g. Obeso 1993), or both (Juenger & Bergelson 1997). In perennials, however, defoliation reduce the probability of flowering for several subsequent years without any perceptible effect on the current seed production (Lubbers & Lechowicz 1989). In the case of orchids, the lack of acute response to defoliation can be explained by efficient translocation of resources from an underground corm (Primack & Hall 1990). However, in many perennial species the store of resources is not sufficient to maintain seed production, and seed maturation may thus be dependent on current photosynthesis (Smith et al. 1986)

Only few studies have assessed the effects of experimental defoliation or herbivory on male function and the results have been very variable. Defoliation has been found to decrease size and/or quality of pollen grains in annual *Raphanus raphanistrum* (e.g. Lehtilä & Strauss 1999), in perennial *Lobelia siphilitica* (Mutikainen & Delph 1996), and in a clonal *Alstroemeria aurea* (Aizen & Raffaele 1996). In another clonal species, *Bromus inermis*, defoliation did not affect pollen production per flower, but it decreased total pollen production via reduced number of flowers (McKone 1989).

1.2.4 Mycorrhizal function

Uptake of nutrients and water from litter and soil may be necessary during flowering even in orchids with large storage organ (Whigham 1984), and this uptake may be affected by the mutualistic plant-fungus interaction (e.g.

Alexander et al. 1984). However, very little is known about the role of mycorrhizal fungi in enhancing uptake of nutrients in adult photosynthetic orchids. The endophytic fungi of orchids utilise cellulose and translocate carbon compounds to protocorms (Hadley 1969), but in autotrophic plants the transport of carbon compounds ceases (Alexander & Hadley 1985). Phosphate has been shown to be transported from mycorrhiza to autotrophic orchids under nutrient stress (Alexander et al. 1984) and mycorrhizal colonisation has been associated with increased concentrations of nitrogen and phosphorus in the tissues of an orchid species, *Goodyera repens* (Alexander & Hadley 1984).

1.3 Subsequent growth and costs of reproduction

Most of studies on plant reproductive success are conducted only on one reproductive season. However, in long-lived perennials, such as orchids, life-time fitness is shared between many reproductive efforts. Life history of plants may be affected both by abiotic (e.g. nutrient and water availability) and by biotic factors, such as herbivores (e.g. Reichman & Smith 1991), which may considerably alter subsequent survival and reproductive plants. Additionally, trade-off between reproductive output and subsequent performance are important for reproductive success of plants (Reekie 1999).

Decreased resource availability caused by e.g. nutrient or water stress, decreased photosynthetic area following defoliation by herbivores or shading, or weakened mycorrhizal function may cause a decrease in stored resources for subsequent seasons (Primack & Hall 1990). A decrease in the amount of stored resources may change the frequency of reproductive efforts related to plant size in orchids. Resource storage may also be crucial for survival of dormant buds during periods of climatic stress (Whigham 1984), and severe depletion of storage be lethal. High fruit set in one year may also decrease growth and reproduction in the following years, since heavy allocation to seed production may deplete resources stored in the underground corm. In orchids, a reduction in the amount of stored resources decreases the probability of flowering or the number of flowers produced in the following year (Snow & Whigham 1989, Ackerman & Montalvo 1990).

1.4 Effects of position on reproductive traits of single flowers

Limited resources cause competition between individuals of the species, but also different parts and functions within a plant compete for both abiotic (e.g. nutrients and water) and biotic (pollinators) resources. The probability of fruit maturation and number or weight of seeds produced are often higher in the first opened flowers than in flowers that are situated farther away from the source of resources and which open later in the season (Diggle 1995). This may

be caused by differences in pollinator behaviour in different parts of an inflorescence, competition among developing fruits for resources, or lower capability of the uppermost flowers to set fruit (Diggle 1995). The size of reproductive structures also tends to decline towards the top flowers due to either architectural constraints or resource limitation (Diggle 1995, Corbet 1998). In addition in female reproductive success, smaller flower size may also decrease the quantity of pollen produced and attractiveness to pollinators (Young & Stanton 1990, Schemske & Ågren 1994).

1.5 Aims of the study

In this thesis, I assessed if reproductive success of terrestrial orchids is mainly limited more by factors related to pollination success than factors related to resource availability, as well as possible interactions between these major factors (Fig. 1). These factors are mainly studied at the level of individual plants, but I also examined how position of a flower within an inflorescence affects its pollination and reproductive success. I specifically address the following questions: (1) Does the frequency of pollinator visitation limit reproductive success of a rewarding and a deceptive terrestrial orchid species (I, II)? (2) In what way does nutrient and water availability and plant size affect reproductive success (I, II)? (3) Does a decrease in photosynthetic area affect plant attractiveness to pollinators, and male and female reproductive traits (III, IV, V)? (4) How important is mycorrhizal function for resource acquisition for seed production (III)? (5) How does resource availability affect subsequent growth and reproduction, and are there costs of reproduction in terrestrial orchids (I-V)? (6) Does the position within an inflorescence affect pollination success, and male and female function of an individual flower (VI)?

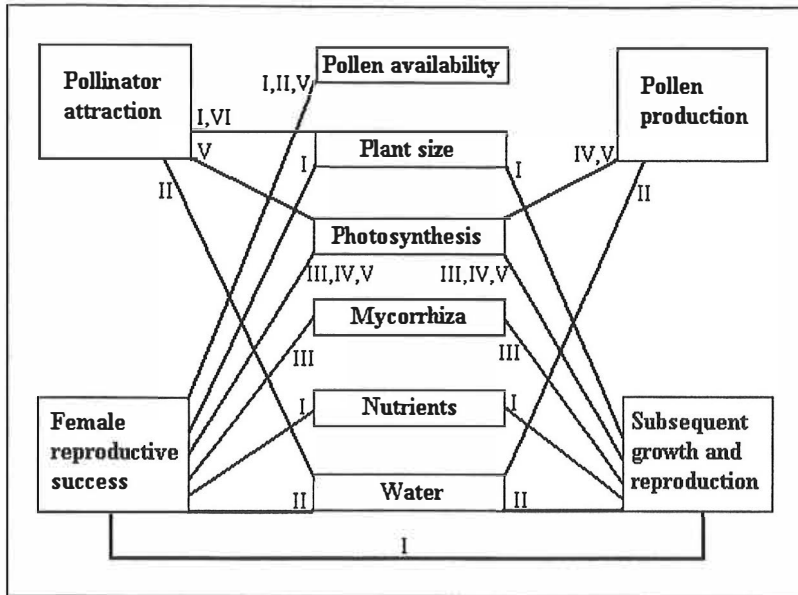


FIGURE 1 Factors that affect reproductive success of terrestrial orchids. Interactions between limiting factors and reproductive traits studied in this thesis are connected with lines. Roman numerals refer to original articles.

2 METHODS

2.1 Study species and sites

Studies of this thesis were conducted natural populations of three terrestrial orchid species: *Dactylorhiza incarnata* (L.) Soó, *D. maculata* (L.) Soó and *Platanthera bifolia* (L.) Rich. in central Finland. Species are long-lived and they store resources in the bulb-like underground corms. Reproduction is fully depended on seed production. In all the species, flowering plants produce a single inflorescence with 5-30 (-60 in *D. maculata*) flowers. Thousands of dust-like seeds are produced in capsules after successful visit of pollen vector. Plants produce a capsule also after self-pollination, but spontaneous autogamy is absent or very rare in nature. Seed germination and development of young plantlets are dependent on presence of mycorrhizal fungi, but performance of autotrophic adult plants is more independent from mycorrhiza.

D. incarnata is a rare threatened species that occurs in mesotrophic and eutrophic sedge fens in southern Finland (Kuitunen & Kuitunen 1994). *D. incarnata* begins flowering begins in June and purple flowers open sequentially during a period of three weeks. The opened flowers remain fresh until almost all flowers are open. The flowers are scentless and contain no nectar (Nilsson 1981, Lammi & Kuitunen 1995). Pollination is based on non-mimic food deception of bumble bee workers (Nilsson 1981). The *D. incarnata* population studied in this thesis is located in the mesotrophic Katajaneva mire in Toivakka (I).

Dactylorhiza maculata is a common species inhabiting moist fertile forests and oxygen- and nutrient-rich mires in Finland (Kuitunen & Kuitunen 1994). *D. maculata* starts flowering in the beginning of June. The flowering individual has an average of 15 pink to red (seldom white) flowers with purple markings. The flowers are born in a spike and they open sequentially. Flowers contain no nectar and pollination is based on non-mimic deception of newly emerged or unconditioned bumble bees (*Bombus* spp.). Species from Empididae, Syrphidae, Halictidae and other insects may also act as pollen vectors (Nilsson 1981). For

this thesis, experiments were conducted in three populations of *D. maculata*. Härkösuo is a small mesotrophic fen with only a few stunted pine trees in Jyväskylä (III, VI). The second population is located on banks of a ditch running from a spring in Laukaa (III, VI). The third population called Väärälampi is a mesotrophic fen located in Konnevesi (IV, VI).

Platanthera bifolia is a common species in Finland that occurs in dry groves, nutrient-rich pine forests, mire edges, and meadows. *P. bifolia* flowers in June–July and produces white flowers with strong sweet scent. The nectar-producing flowers are visited by noctuid long-tongued moths (Sphingidae, Noctuidae) (Nilsson 1981). This species was studied in two different populations, both of them in mixed birch and pine forests (I, II, V).

2.2 Pollen and resource availability

2.1 Pollen availability

To examine whether the species studied were pollination-limited, female reproductive success of naturally pollinated (open-pollination) and experimentally hand-pollinated plants was compared. In hand-pollination each flower of the experimental plants was pollinated with one pollinium taken from an other plant in the same population (cross-pollination). Pollination success of the experimental plants was estimated as the percentage of flowers initiating capsules (*D. incarnata*, *P. bifolia*; I, II), the relative capsule production, or the dry weight of capsules (*P. bifolia*; V).

2.1.1 Resource availability

The availability of nutrients was experimentally increased by fertilising *D. incarnata* and *P. bifolia* plants with organic fertiliser sticks (I). Water stress of *P. bifolia* was studied in a summer of low precipitation (1997) by watering the experimental plants regularly during the flowering (II). The effects of photosynthetic ability on attractiveness to pollinators and reproductive traits were studied in defoliation experiments with *D. maculata* and *P. bifolia*. Either a half of each leaf (IV, V) or all the leaves (III, IV, V) were cut off prior to flowering. The effects of photosynthetic area on seed production were further studied with *D. maculata* by shading the upper part of the plant (stalk and inflorescence) with a dark green, tightly woven cloth bag (III).

The effect of mycorrhizal function on reproductive success was studied with *D. maculata* (III). Mycorrhizal function was inhibited by treating the roots of the experimental plants with fungicide (thiabendazole) solution. Thiabendazole reduces colonisation and growth rate of mycorrhizal fungi in the roots of orchids, but it has no phytotoxic effects (Alexander & Hadley 1984). The solution used in the experiment was made according to the procedure

presented in Alexander & Hadley (1984) and the dose was modified to function *in situ*.

2.3 Attractiveness to pollinators and reproductive success

2.3.1 Plant size and flower characters

In all studies, the experimental plants were measured to estimate their vigour and the amount of resources stored in the underground corm. The height was measured with a ruler from the base of the stalk to the top of the inflorescence, and the number of flowers was counted. To determinate the leaf area of the experimental plants, the length (L) and the maximum width (W) of rosette leaves were measured. These measurements were used to calculate leaf areas (A) of individual plants of different species using the following linear regression equations:

$$D. \textit{incarnata}: A = L \times W \times 2/3 \text{ (I)}$$

$$D. \textit{maculata}: A = 19.85 \times \log(L) + 19.52 \times \log(W) - 11.30 \text{ (III, IV)}$$

$$P. \textit{bifolia}: A = 1.05 \times L + 6.62 \times W - 12.26 \text{ (I, II, V)}$$

The first equation has previously been used for calculating of leaf area in *D. incarnata* (Lammi & Kuitunen 1993). For the latter two equations leaves were collected from *D. maculata* and *P. bifolia* plants, the length and width of plants were measured with a ruler and actual leaf area was measured with graph paper. With these measurements the best possible equation to calculate leaf area was searched with regression analysis.

To be able to evaluate the attractiveness of flowers to pollinators, spur length and height of nectar column of *P. bifolia* plants were measured using digital caliper with 1.0 mm accuracy (V). Flower size of *D. maculata* was estimated by measuring both the length and maximum width of labellum and the spur length in three equal-sized sections of the inflorescence (lowermost, middle and uppermost) using a digital caliper (VI).

2.3.2 Female and male reproductive traits

Female reproductive success of the experimental plants was measured as capsule initiation (total number of capsules/number of flowers), relative capsule production (mature capsules/number of flowers) and dry weight of capsules (oven-dried at 80°C, 24 h). The proportion of seeds with well-developed embryo of all seeds was determined using samples of 100-500 seeds that were studied under a microscope. The relationship between the capsule dry weight and the number of seeds in it was calculated for *D. maculata* (III) and *P. bifolia* (II). It was found that the capsule weight is a good estimate for total seed production and for the production of embryonic seeds in both species.

Allocation of resources to male reproductive functions was studied in *D. maculata* (IV, VI) and *P. bifolia* (II, V). Pollinia were collected from fresh flowers with the toothpick and preserved in a freezer. The frozen pollinia were removed from the toothpick with tweezers and weighed with a microbalance. Pollen quality of differentially defoliated *D. maculata* plants was studied by pollinating six recipient flowers in 15 non-experimental plants (IV). Two flowers of each recipient plant were pollinated with one pollinium from the non-defoliated, partially defoliated or completely defoliated plants. Differences in quality of pollen was estimated both as the dry weight of capsules and as the proportion of embryonic seeds produced by flowers within the same inflorescence pollinated with pollen from the differentially treated donor plants.

2.3.3 Subsequent growth and reproduction

The effects of resource manipulations and previous capsule production on subsequent growth were studied by measuring leaf areas of the experimental plants in the year following the treatments (I – V). In the defoliation experiment with *D. maculata* (IV) corms of 18 plants were dug up, oven-dried (80°C, 24h) and weighed. Effects of the treatments on subsequent reproduction was measured as the proportion of plants that produced inflorescence in each treatment group (I – IV). Reproductive success of plants in the following year was not tested, because there were too few flowering plants in some of the treatment groups.

2.4 Data analyses

Statistical tests were performed with SPSS for Windows and Statistix. The data were tested with factorial and one-way analysis of variance and t-test. Leaf area in the year of treatments was used as a covariate. Non-parametric tests were applied when the assumptions of parametric tests were not met. Statistical tests are described in more details in papers I-VI.

3 RESULTS AND DISCUSSION

3.1 Pollination limitation (I, II, VI)

Hand-pollination did not affect capsule initiation in the nectar-producing *P. bifolia*, but it increased the production of mature capsules (I). In the year of poor water availability (1997), hand-pollination increased both capsule initiation and production of mature capsules in watered plants, but had no effect on female reproductive success in non-watered plants (II). *P. bifolia* plants with larger inflorescence produced relatively more mature capsules than smaller plants (I). These results suggest that nectar production ensures pollinator visitation and thus capsule initiation in *P. bifolia*, but production of mature capsules may be limited by both insufficient amount of pollen deposited on stigmas and resource availability. Watering increased the amount of nectar produced, but it did not affect capsule production in open-pollinated *P. bifolia* plants (II). Defoliation did not affect spur length, nectar production or proportion of flowers opened during flowering, but it decreased the relative capsule production in the open-pollinated population (V). Resource availability and pollination success also seem to have a more straightforward interaction, because poor resource situation may decrease a plant's attractiveness to pollinators via e.g. decreased odour.

Hand-pollination of all flowers with cross-pollen increased both capsule initiation and production of mature capsules in *D. incarnata*. Therefore, within-year reproductive success of the this nectarless species seems to be purely pollination-limited as suggested by many other studies with orchids (see Nilsson 1992). However, inflorescence size did not affect relative capsule production either in *D. incarnata* or in *D. maculata*. Lammi (1999) also found that relative female reproductive success of *D. incarnata* was not dependent on the inflorescence size. So, there seems to be no advantage of large inflorescence size in pollinator attraction in these species. However, plants with a large inflorescence have longer flowering times, and therefore large plants may produce absolutely more seeds than smaller plants. Nilsson (1992) also argued

that nectarless plants with large inflorescences may have smaller probability to be left without pollinator visits. Pollination success of nectarless species may be more dependent on random pollinator movements than interplant differences in traits related to pollinator attraction i.e. inflorescence size.

3.2 Plant size and nutrient availability (I)

Nutrient application increased the production of mature capsules in small *P. bifolia* plants, but had no such effect in large plants. Small plants were thus more dependent on current nutrient availability than large plants, probably due to insufficient store of resources in the underground corm. To my knowledge, this is the first study showing positive effects of nutrient application on performance of adult terrestrial orchids. However, Whigham (1984) suggested that the need for macronutrients may exceed the amount stored in the underground corm in the terrestrial orchid, *Tipularia discolor*, and plants have to assimilate nutrients from the soil at the time of growth and reproduction.

Increased nutrient availability did not affect capsule production in the nectarless *D. incarnata*. There was also no difference in relative female reproductive success between plants with different sized inflorescences. The fact that nutrient application had no effect on female traits may be due to uncertainty in pollinator visitation which may lead to "bet-hedging" (Ayre & Whelan 1989), i.e. producing of as many seeds as possible in the case of good pollination success despite possible negative effects on survival and subsequent reproduction.

Both *P. bifolia* and *D. incarnata* plants with larger leaves in the previous year had a greater probability of flowering in the following year. *P. bifolia* plants with larger inflorescence had a greater possibility to persist to the stage of capsule maturation in the year of low precipitation, while some of the smaller plants withered before the end of flowering. In terrestrial orchids, plant size has been found to relate to the amount of stored resources (Fritz 1995, Alexandersson & Ågren 1996), and reproductive success of a plant may depend on its ability to store resources before flowering (Calvo 1990).

3.3 Water availability (II)

Watering did not affect capsule initiation in non-watered plants, but it increased the production of mature capsules in hand-pollinated *P. bifolia* plants. These results suggest that water availability could not limit female reproductive success at normal pollinator visitation rate. Similarly, another terrestrial orchid species, *Tipularia discolor*, produced less fruits after hand-pollination in a dry year than in a normal year, but in control plants there was no difference in fruit production between years (Snow & Whigham 1989).

Watering did not affect the weight of pollen produced by *P. bifolia* flowers. This may be a result of a better resource situation at the beginning of the flowering season when the pollinia mature. These results may also indicate contrasting strategies of the male and female components of a hermaphroditic plant. In conditions of poor resource availability, it may be profitable for a plant to allocate more to the energetically cheaper pollen instead of the costly female functions (Smith & Evenson 1978). Watering increased nectar production of *P. bifolia*, but watered open-pollinated plants did not have relatively more pollinations than control plants. There was thus no secondary effect on female reproductive success, but pollen export may have increased with increasing reward, which has been found in other plant species (Stanton & Preston 1988, Real & Ratchke 1991, Hodges 1995).

3.4 Availability of photosynthates (III, IV, V)

Complete defoliation (removal of all the leaves of a plant) decreased relative capsule production in one of the two populations of *D. maculata*, and it decreased the dry weight of capsules in both populations (III, IV). A decrease in photosynthetic capability has been shown to cause a reduction in seed number also in other perennial plants (e.g. Lehtilä & Syrjänen 1995). Defoliation had no effect on the proportion of embryonic seeds produced. Shading of inflorescence decreased relative capsule production in the other population of *D. maculata*, and it decreased dry weight of capsules in both populations. This effect was similar to the decrease in seed set following defoliation. Therefore there seems to be no difference in the importance of photosynthates from rosette leaves and those from the green parts of the inflorescence. In contrast to this result, Primack & Hall (1990) suggested that photosynthates for seed production in the terrestrial orchid species *Cypripedium acaule* originate mainly from flower stalk and fruit surface, in addition to resources originating from the rhizome.

Defoliation did not affect the weight of pollinia either in *D. maculata* or in *P. bifolia*. Defoliation had no effect on the quality of pollen produced in *D. maculata*; there was no difference in the dry weight or in the proportion of embryonic seeds produced between flowers pollinated with pollinia from defoliated plants and from non-defoliated plants. The fact that male reproductive traits were not affected may be due to an advantage in timing of pollen maturation compared to female functions. Defoliated plants may also have allocated relatively more resources to pollen production than to the more costly female function.

There were no differences in spur length or nectar production between non-defoliated, partially defoliated, and completely defoliated *P. bifolia* plants. Moreover, defoliation did not affect the number of opened flowers. These results indicate that the characters related to pollinator attraction may be well buffered against changes in resource availability. However, in the open-pollinated population, completely defoliated *P. bifolia* plants produced relatively less and lighter capsules than non-defoliated plants (V). According to

other studies in this thesis (I, II), a decrease in resource availability decreases capsule weight and thus seed set, but does not affect relative capsule production. Therefore it seems likely that the decrease in relative capsule production following defoliation observed in the open-pollinated population could be caused by decreased pollinator attraction. Decreased resource availability, similar to defoliation, has been found to cause a decrease in scent production in *P. bifolia* (Tollsten 1993). It is thus possible that diminished capsule production following defoliation is caused by decreased scent.

3.5 Mycorrhizal function (III)

Inhibition of mycorrhizal function did not affect relative capsule production in *D. maculata*, but capsules produced by plants treated with thiabendazole were lighter in weight than those produced by non-treated plants in one of the two populations. The differences in response to thiabendazole treatment between the populations may be due to different nutrient status. Hadley & Pegg (1989) found no differences in growth and function between infected and uninfected *Dactylorhiza majalis* plantlets growing in conditions with good nutrient availability. However, under nutrient stress the movement of phosphate into roots of *Goodyera repens* has been found to be greater in infected than in uninfected plants (Alexander et al. 1984).

3.6 Subsequent growth and costs of reproduction (I-V)

Generally, increased availability of resources is found to enhance survival, and subsequent growth and reproduction of plants. However, nutrient application and watering treatment did not affect subsequent leaf area or probability of flowering in *P. bifolia*. Additionally, an increase in nutrient availability resulted in decreased leaf area in *D. incarnata* in the year following treatment. Different responses of these species may be due to a difference in importance of mycorrhizal function. *P. bifolia* seem to be non-sensitive to changes in mycorrhizal interactions, because small plants were able to directly utilise increased nutrient availability. Additionally, a possible increase in root competition with other species may not be as harsh in this *P. bifolia* population as in more dense habitats such as meadows. In contrast, mire species, such as *D. incarnata*, may be more dependent on mycorrhizal function than the orchid species occurring for example in forests. However, inhibition of mycorrhizal function in another mire species, *D. maculata*, did not affect leaf area or probability of flowering in the following year. A decrease in subsequent leaf area following nutrient application in *D. incarnata* may also be due to increased interspecific root competition with neighbouring sedges.

Defoliation treatments did not affect subsequent leaf area in *P. bifolia*. However, complete inhibition of photosynthesis by defoliation and shading treatments resulted in decreased leaf area in *D. maculata* in the following year. In this species also the corm mass was found to decrease after defoliation, indicating that the plants allocate to reproduction despite severe depletion of stores in the underground corm. Whigham (1990) found that leaf area and corm biomass in *Tipularia discolor* were reduced in the year following complete defoliation. Probability of flowering was not affected by defoliation and shading treatments in one of the two populations of *D. maculata* (Laukaa, III), but in an other experiment, completely defoliated plants had lower probability of flowering in the following year when compared to non-defoliated plants (IV). The lack of effect of defoliation on subsequent reproduction in Laukaa may be due to differences in light and nutrient availability. In Laukaa, the *D. maculata* population is on banks of a ditch heavily shaded by a thick canopy of spruces and alders. The reproductive success of plants in this population also seemed to be more dependent on mycorrhizal function than in an other population examined in the study (III).

Heavy capsule set following hand-pollination did not affect the probability of flowering in the following year in *P. bifolia*, but in *D. incarnata* the plants with high relative capsule production had lower probability of producing flowers in the following year. These results suggest that in *P. bifolia*, nectar production ensures pollinator visitation and reproductive success may vary less between the seasons. In contrast, relative pollination success of the nectarless *D. incarnata* may vary between 0% and 100% depending on the availability of naive pollinators and their unpredictable behaviour. Therefore, it may be more profitable in terms of life-time fitness of a nectarless species to produce the maximum number of seeds in case of good pollination success, although future reproduction may be delayed or even prevented.

3.7 Position-dependent reproductive success of flowers (VI)

In *D. maculata*, the probability of a flower to have pollinations was independent on its position in inflorescence. Flower characters did not seem to affect pollinator visits, either because the probability to produce capsules did not differ between the smaller flowers in the uppermost part of the inflorescence and the larger flowers in the lowest positions. This result supports the earlier finding that naive pollinators do not discriminate between inflorescence characters, and pollination of this nectarless species is more or less a fortuitous event. The dry weight of capsules, however, decreased towards the top of the inflorescence both in open-pollinated and in hand-pollinated plants. When flowers from the lowermost and middle parts of the inflorescence were removed capsule weight in the top of the inflorescence did not differ from capsules produced by the lowermost flowers of control plants. These results indicate that also the uppermost flowers are capable of producing large capsules and thus seed production of upper flowers seems to be limited by

competition for resources with flowers in lower positions. Flowers of the nectarless *D. maculata* stay fresh for a long time if not pollinated, and therefore the maintenance of flower structure and pollen viability may extract a significant part of available resources before the opening of the top flowers. Pollination with self-pollen resulted in lower seed set than pollination with cross-pollen, but there was no difference in the extent to which seed production decreased after geitonogamous pollination between the three sections of the inflorescence. Avoidance of geitonogamy may, however, not limit number of flowers in *D. maculata*, because pollinator visits in its nectarless inflorescence of orchids have been found to be very short and therefore risk of geitonogamous pollination is low (Johnson & Nilsson 1999).

CONCLUSIONS

In nectar-producing orchid species pollination is ensured by pollinator reward, while the pollination success of nectarless species is very variable and dependent on unpredictable pollinator movements. Attractiveness to pollinators may, however, be affected by other factors, for example defoliation by herbivores also in rewarding species. The female reproductive success of nectar-producing species is more dependent on current resource availability, while nectarless species produce seeds using resources provided mainly by storage in the underground corm. The function of mycorrhizal fungi was also found to play a part in seed production of *D. maculata*, but the importance of mycorrhiza to reproductive success may vary between the populations. A decrease in photosynthetic area does not cause abortion of seed capsules in terrestrial orchids, but defoliated plants produce less seeds per capsule than non-defoliated plants. However, male reproductive traits were not affected by defoliation either in nectar-producing or in nectarless species. Male function precedes female function during the reproductive period, and therefore there may be more resources available for male than female traits. Size of a plant limits reproductive success especially in nectar-producing species, because large plants have more stored resources and they are more tolerant to harsh conditions during the flowering. Nectarless plants with large inflorescence do not have better pollination success. Large number of flowers, however, mean longer flowering time and thus increase in the probability of successful pollination. Additionally, surplus-flowers may serve as ovary reserve if some of the flowers are lost during the reproduction. Capsules produced in the top of the inflorescence also contain hundreds of seeds even though they are smaller than capsules produced in the base of the inflorescence due to resource competition between the flowers. High relative capsule production does not affect the leaf area and the probability of flowering in nectar-producing species, but in nectarless species there is cost of reproduction. This difference in cost of reproduction may be due to different reproductive strategies. Reproductive success is more or less constant in rewarding species, and therefore plants with moderate seed set in consequent years may have the highest life-time fitness. On the contrary, in nectarless species pollination success may vary between the years and it is advantageous for a plant to produce as much seeds as possible when pollinator success is good, even if heavy seed set may considerably delay or weaken subsequent reproductive effort.

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YHTEENVETO

Kämmeköiden lisääntymismenestystä rajoittavat tekijät

Tutkin väitöskirjatyössäni eurooppalaisten maakämmeköiden lisääntymismenestykseen vaikuttavia tekijöitä. Pölytysten saantia pidetään yleisesti tärkeimpänä kämmeköiden lisääntymismenestystä rajoittavana tekijänä, koska monilla lajeilla on hyvin erikoistuneita pölytysstrategioita. Maakämmekät muistuttavat kuitenkin enemmän muita ruohovartisia monivuotisia lajeja kuin trooppisina päällyskasveina kasvavia sukulaisiaan, joten niiden lisääntymismenestys voi olla myös muiden tekijöiden kuin pölytysten saannin rajoittamaa. Tutkimuslajeikseni valitsin kaksi pölyttäjiään pettävää, medetöntä kämmekkälajia, Keski-Suomessa uhanalaisen punakämmekän (*Dactylorhiza incarnata*) ja sen yleisen lähisukulaisen maariankämmekän (*Dactylorhiza maculata*), sekä medellisen, yöperhosten pölyttämän valkolehdokin (*Platanthera bifolia*).

Väitöskirjatyöni osatutkimuksissa I ja II olen selvittänyt resurssilisäyksen vaikutusta punakämmekän ja valkolehdokin lisääntymiseen. Lannoitin punakämmekkä- ja valkolehdokkiyksilöitä ravinnepuikoilla (I) ja kastelin valkolehdokkiyksilöitä kuivana kesänä (II) sekä seurasin näiden koeyksilöiden kotamuodostusta sekä luonnollisesti pölyttyneissä että käsin 100%:sti pölytetyissä kasveissa. Ravinnelisäyksellä ja kasvin koolla ei ollut vaikutusta medettömän punakämmekän kotatuottoon. Valkolehdokilla sen sijaan pienten yksilöiden siementuotto lisääntyi ravinnelisäyksen jälkeen. Käsipölytys puolestaan lisäsi punakämmekän kotamuodostusta, mutta se ei vaikuttanut valkolehdokin naaraspuoliseen lisääntymismenestykseen. Kastelu lisäsi kotatuottoa vain käsipölytetyillä valkolehdokeilla. Nämä tulokset osoittavat, että medettömän punakämmekän lisääntymismenestys on satunnaisten pölyttäjävierailujen rajoittamaa, kun taas valkolehdokin lisääntymismenestys on riippuvaista sekä pölytysten saannista (yksilön houkuttelevuus pölyttäjille) että resurssien (ravinteet, vesi) saannista. Suuri siementuotto edellisellä vuonna ei vaikuttanut valkolehdokin todennäköisyyteen kukkia seuraavana vuonna, mutta medettömän punakämmekän kukintatodennäköisyys aleni voimakkaan

lisääntymispanostuksen seurauksena. Medellisenä lajina valkolehdokin vuosittainen siementuotto on varmempaa kuin medettömän punakämmekän, joka pyrkii tuottamaan mahdollisimman paljon siemeniä onnistuneiden pölytysten jälkeen, vaikka sillä olisikin negatiivinen vaikutus lisääntymismenestykseen tulevaisuudessa.

Osatutkimuksissa III-V selvitin yhteyttämistehokkuuden vaikutusta lisääntymismenestykseen maariankämmekällä ja valkolehdokilla. Defoliaatio-kokeissa kasvin lehdistä poistettiin puolet tai ne poistettiin kokonaan ennen kukinnan alkua, mikä vähensi yhteyttämispinta-alaa ja siten kasvin käytössä olevan energian määrää. Defoliaatio ei vaikuttanut kasvien siitepölyn tuottoon eikä kotia muodostaneiden kukkien suhteelliseen osuuteen, mutta defolioitujen kasvien kodat olivat kevyempiä ja sisälsivät vähemmän siemeniä kuin käsittelemättömien kasvien kukat. Lehtien poistaminen ei vaikuttanut valkolehdokin kukkien kannuksen pituuteen eikä meden tuottoon, mutta defolioidut kasvit tuottivat kuitenkin vähemmän kotia kuin kontrollikasvit, mikä voi johtua huonontuneen resurssitilanteen vaikutuksesta esim. pölyttäjiä houkuttelevan tuoksun tuottoon. Myös varressa ja kukinnan vihreissä osissa tapahtuva yhteyttäminen sekä mykoritsasienen toiminta vaikuttavat siementuottoon maariankämmekällä, mutta tärkeimmäksi resurssien lähteeksi havaittiin maanalainen varastajuuri. Varastajuureen kertyneiden resurssien määrään vaikuttavat kuitenkin myös esim. yhteyttämisen tehokkuus, joten resurssien saatavuus vaikuttaa lisääntymismenestykseen myös epäsuorasti kasvin koon ja kukintatodennäköisyyden kautta.

Maariankämmekän kukkien koko, tuotetun siitepölyn määrä ja kotien kuivapaino laskivat alimmista kukista kukinnan latvaa kohti. Kukkiensa koko ja siitepölyn tuotanto voivat olla riippuvaisia kukinnan kehityksen aikana ns. rakenteellisista vaikutuksista kukinnan kasvun aikana, mutta ylimpien kukkien siementuottoa rajoitti kilpailu resursseista alempien kukkien kanssa. Suuresta kukinnosta voi olla hyötyä medettömälle lajille erityisesti kukinta-ajan pitenemisen vuoksi. Suuri kukkamäärä mahdollistaa suuren kotatuoton, jos kasvi saa hyvin pölytyksiä. Latvakukat voivat toimia myös ns. lisääntymis-reservinä, jos alemmat kukat tuhoutuvat kukinnan aikana.

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I

Nutrient versus pollination limitation in *Platanthera bifolia* and *Dactylorhiza incarnata*
(Orchidaceae)

by

Elisa Mattila and Markku Kuitunen, 2000

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II

The effects of water stress and pollen availability on reproductive success of *Platanthera bifolia* (Orchidaceae)

by

Elisa Mattila , 2000

Det Norske Videnskaps-Akademi. I. Matematisk-Naturvidenskapelige Klasse.
Avhandlingar. Ny Serie. (in press)

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III

Sources of resources for seed production in *Dactylorhiza maculata* (Orchidaceae)

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IV

Effects of defoliation on male and female reproductive traits of a perennial orchid,
Dactylorhiza maculata

by

Elisa Mattila and Veikko Salonen

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V

The effect of defoliation on attractiveness to pollinators and reproductive success in
Platanthera bifolia (Orchidaceae)

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VI

Position-dependent reproductive success of flowers in *Dactylorhiza maculata*
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