

Antti Lammi

Reproductive Success,
Local Adaptation and Genetic Diversity
in Small Plant Populations



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ABSTRACT

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Diss.

Most scientists agree that both ecological and genetic processes combined with stochasticity will largely determine the future of a certain population. In this thesis I examine some of these ecological and genetic factors in three vascular plant species, each of them occurring in small and isolated populations. Deceptive pollination system of *Dactylorhiza incarnata* was found to function best in remote habitats like mires where there are no concurrently flowering plants attractive to the pollinators. However, individual (e.g. floral display) and other population characteristics (e.g. size and detectability) seemed to have only secondary importance for male and female reproductive success. The amount of pollinia received was found to be more important for the reproductive success of *D. incarnata* than the genetic quality and the origin of pollinia.

The importance of local adaptation in the relationship between an annual, endangered root hemiparasite *Euphrasia rostkoviana* and its host, *Agrostis capillaris*, was studied in a pot experiment. After three months of growth, all variables measured for hemiparasite performance during its complete life cycle gave only limited support for the local adaptation hypothesis.

The level of genetic diversity was found to vary substantially among isolated and small populations of *Lychnis viscaria*. In general, genetic diversity in the populations was low. There was limited gene flow and high differentiation among populations. The peripheral populations of *L. viscaria* had less genetic variation than the central ones. Population size was positively correlated with genetic diversity. Population size and genetic diversity, however, were not associated with individuals' fitness components such as germination rate, seedling mass or seed yield. There were no differences in the measured fitness components between peripheral and central populations. Even though small and peripheral populations had lower level of genetic variation, they were as viable as larger populations. These results give support for the demands of directing conservation practises not only to large central populations but to small, isolated and peripheral populations as well.

Key words: *Dactylorhiza*; deceptive pollination; *Euphrasia*; genetic diversity; hemiparasites; isolation; local adaptation; *Lychnis*; orchids; pollinators.

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List of original publications

This thesis is based on the following publications and manuscripts, which will be referred to in the text by their Roman numerals. I have personally planned, performed a significant proportion of the work and written the papers I-IV. In paper V, I have planned and performed a significant proportion of the work and I have been the main writer of the paper.

- I Lammi, A. & Kuitunen, M. 1995. Deceptive pollination of *Dactylorhiza incarnata*: an experimental test of the magnet species hypothesis. *Oecologia* 101: 500-503.
- II Lammi, A., Mattila, E. & Kuitunen, M. 1999. Reproductive success in nectarless *Dactylorhiza incarnata*: the effects of attractive floral display and population detectability. Manuscript submitted.
- III Lammi, A., Mattila, E., Vauhkonen, T. & Kuitunen, M. 1999. Outcrossing, hybridization, pollen quantity and the evolution of deceptive pollination in *Dactylorhiza incarnata*. Manuscript submitted.
- IV Lammi, A., Siikamäki, P. & Salonen, V. 1999. The role of local adaptation in the relationship between an endangered root hemiparasite *Euphrasia rostkoviana*, and its host, *Agrostis capillaris*. *Ecography* 22: 145-152.
- V Lammi, A., Siikamäki, P. & Mustajärvi, K. 1999. Genetic diversity, population size and fitness in central and peripheral populations of a rare plant *Lychnis viscaria* (Caryophyllaceae). *Conservation Biology* (in press).

1 INTRODUCTION

Although we most often think the greenhouse effect when the phrase global change is used, human activities are changing the world in another, equally profound way. Our activities dominate many of the world's ecosystems. Fewer and fewer ecosystems escape our influence. The current activities are creating islands of natural ecosystems in a sea of human-dominated ones (Holsinger 1993). During the last centuries there has been a dramatic decline in biological diversity. For instance, the estimated number of endangered plant species worldwide is 60 000 and in several areas plants dominate the lists of endangered species (Fenster & Dudash 1994). Nearly 25 % of the estimated 250 000 species of vascular plants in the world may become extinct within the next 50 years (Raven 1987).

In general, a major threat against many endangered species is the increasing fragmentation of their habitats, which may have a dramatic impact on both the ecological and evolutionary dynamics of the species (Holsinger 1993). At some spatial scale, most plant species are patchily distributed due to their sedentary habit and the spatial heterogeneity of the environment. Patchy distributions have been further exaggerated by the activities of humans through habitat destruction and fragmentation (Olesen & Jain 1994, Schemske et al. 1994, Leimar & Nordberg 1997). Thus, natural populations of rare, endangered and threatened species are typically geographically isolated (e.g. Ellstrand 1992, Fenster & Dudash 1994). Isolated populations are often small as well which, for example, may have severe consequences for reproduction and therefore demography of populations. The intuitive feeling that a rarer species is more prone to extinction has been supported both by theoretical studies and empirical data. Both demographic models (e.g. Goodman 1987, Lande 1988, Pimm et al. 1988) and field data (Diamond 1984, Pimm et al. 1988) have shown that the probability of extinction of a local population declines as its size increases (Arita et al. 1990, Ellstrand 1992).

What are the factors involved in the extinction and survival processes of natural populations? Most scientists agree that both ecological and genetic processes combined with stochasticity will largely determine the future of a certain

population (Schemske et al. 1994, Vrijenhoek 1994). However, the relative importance of these factors is still under debate (e.g. Boyce 1992, Fenster & Dudash 1994, Schemske et al. 1994, Saccheri et al. 1998). An ecological approach emphasizes the biotic interactions and habitat requirements of species, whereas genetic approach has stressed that understanding the organization of genetic diversity is a key to the long-term survival of species (e.g. Lande 1988, Schemske et al. 1994).

The ecological factors shown to influence plant population viability include e.g. environmental variability and interspecific interactions (e.g. Schemske et al. 1994). In the case of animal pollinated plant species, pollinators may have an important role in determining reproductive success and consequently, population viability (Kearns & Inouye 1997, Sipes & Wolf 1997). Especially in outcrossing and self-incompatible plants, reproductive success is completely determined by animal vectors required to transfer pollen among individuals. Pollination success may be highly important in self-compatible species as well, such as *D. incarnata*, if populations are small in size (e.g. Powell & Powell 1987, Rathcke & Jules 1993, Ågren 1996).

Local adaptation may also have an important role for ensuring population persistence (Lesica & Allendorf 1995). Some plants can experience natural selection on a very fine spatial scale due to their sessile habit and restricted pollen and seed dispersal (e.g. Schmitt & Gamble 1990). Local adaptation may manifest itself e.g. in interspecific competition or in parasite-host interactions. It can be predicted, that the hemiparasitic plants may be locally adapted to their hosts, which has consequences for population persistence.

Genetic diversity, population structure and gene flow could have a major role in determining the fitness of patchy and isolated populations. The role of genetic variation is emphasized, since genetic variation is a requisite for evolutionary adaptation. Genetics may have short-term fitness consequences (Huenneke 1991, Schemske et al. 1994), which may have a great impact on population viability and persistence.

In this thesis I examine these ecological and genetic factors in small populations of three vascular plant species (*D. incarnata*, *E. rostkoviana*, *L. viscaria*), occurring in marshes or semi-natural grasslands. I use these species as examples to focus on the different aspects of this challenging issue. Based on my results, I conclude this thesis by evaluating the importance of different approaches to plant conservation.

1.1 How to get pollinators without rewards ? - the determinants of reproductive success in small populations of *Dactylorhiza incarnata* (I, II)

(I) Most insect-pollinated plant species attract their pollinators with rewards, usually nectar or pollen or both. Nevertheless, some species do not offer rewards and yet they are pollinated by insects. These species have deceptive pol-

lination systems that are supposedly derived from reward-pollination systems, without the loss of reproductive success (Ackerman 1986, Nilsson 1992). Many fraud orchids exploit pollinator foraging behavior by attracting insects with a variety of dummy signals and nectarless or pollenless structures.

Nectarless *D. incarnata* is apparently adapted to exploit short visits by inexperienced or unconditioned bumble bee workers (Daumann 1941, Nilsson 1981, Fritz & Nilsson 1994) which find their first food-flowers solely by optical cues. According to Nilsson (1981) the deception system will function best in remote locations such as mires, where there are no concurrently flowering plants attractive to the pollinators and even a nectarless species may gain enough pollinations. An alternative theory predicts that nectarless species take advantage of nectar-containing, co-blooming species. According to this "magnet-species theory" nectarless plants receive more pollinators at the expense of the nectar-containing species (Thomson 1978, Rathcke 1983, Lavery & Plowright 1988, Lavery 1992). Thus, the quality of a habitat, in terms of pollination success, could contribute to the long-term survival of species.

(II) In some open habitats, like fen-mires, the pollinators presumably can enhance foraging efficiency by making direct visual comparisons between competing individual plants, by flying not only to the closest ones, but also to the ones with the largest floral display (Heinrich 1983). The largest displays are favored because of the expected positive correlation between display size and reward (Cohen & Shmida 1993). Investment in display may thus pay off in fitness through either male (pollen donation) or female (fruit production) sexual functions or both.

It has been suggested that in pollinator limited plants attractiveness should increase pollen export (e.g. Stanton & Preston 1988, Queller 1997). One would predict, that especially in nectarless orchids large floral display size should increase male fitness, since most nectarless orchids have no other means to attract pollinators. Attractiveness may affect female reproductive success as well, but the patterns are not always clear. Female reproductive success (fruit set) has been shown to correlate positively with floral display size in some nectarless orchids (e.g. Nilsson 1980, Fritz 1990, Pettersson & Nilsson 1993, Fritz & Nilsson 1994, 1995), while other nectarless orchids have not shown the same pattern (Montalvo & Ackerman 1987, Firmage & Cole 1988, Zimmerman & Aide 1989).

Moreover, the variation in display size within population could make the learning process of pollinators more difficult, thereby improving the reproductive success of nectarless orchids (e.g. Sabat & Ackerman 1996). It could be that the floral display size may not be such an important factor affecting pollination success as it is in species with nectar.

In principle, population size could be one factor affecting reproductive success and viability of populations. In nectar-containing species pollination success has been observed to decrease in smaller populations and in less dense patches (for reviews see Rathcke & Jules 1993, Olesen & Jain 1994, Kearns & Inouye 1997). In contrast, Stoutamire (1971) suggested, that the avoidance learning by bees might prevent some nectarless orchids from developing large populations.

Also, the location of a population, which together with size determines its detectability, may strongly affect pollinator visitation rates and reproductive success (Firmage & Cole 1988, Cohen & Shmida 1993, Alexandersson & Ågren 1996). Altogether, reproductive success may decline in the most remnant and smallest populations with little visual attraction.

Population characteristics, such as population size and location, may also affect the ratio of male and female reproductive success within populations. The departures from hypothetical equal pollinium removal and fruit set in populations may reflect different pollen wastage movement patterns of the pollinators. The ratio between male and female reproductive success and attractiveness provides valuable information of the efficiency of pollination system, which may have important evolutionary consequences (Fritz & Nilsson 1994).

1.2 How has deceptive pollination evolved? - the importance of pollen quality and quantity in *Dactylorhiza incarnata* (III)

Evolution of deceptive pollination systems is enigmatic and there are several hypotheses explaining this development. All of these hypotheses agree that the lack of pollinator reward reduces pollinator activity and that the reduction of pollinator visitation must be outweighed by other advantages (Montalvo & Ackerman 1987, Ackerman & Montalvo 1990, Nilsson 1992). The "outcrossing" hypothesis proposes that greater outcrossing is achieved by longer flight distances of deceived pollinators between successively visited flowers (e.g. Heslop-Harrison 1968, Nilsson 1992, Peakall & Beattie 1996). The "outcrossing" hypothesis emphasizes the importance of pollen source and genetic quality for reproductive success. The hypothesis is based on the idea that inbreeding depression is always occurring either through selfing or biparental inbreeding, and it ignores the possibility for outbreeding depression over the range of flight distances of deceived pollinators. The hypothesis also assumes that genetic distance between plants is related to a physical distance (e.g. Waser & Price 1983, 1993, Hauser & Loeschcke 1994).

In the deceptive orchid *D. incarnata*, crossing distance is related to hybridization as well. Populations of *D. incarnata* are usually located in small open mires surrounded by herb-rich swamp woods inhabiting the closely related nectarless species *Dactylorhiza maculata* (L.) Soó (Lammi & Kuitunen 1995). These species have similar floral display and there is often a hybrid zone around open mires in which hybrids and pure species coexist. Thus, the benefit of greater crossing distance could be lost if the probability of hybridization increases with increasing crossing distance.

The evolution of deceptive pollination has also been explained by the "pollinia" hypothesis, which proposes that pollinia (the packaging of a plants' pollen into a few separate units) allow such a great pollination success from single visits that no reward to attract pollinators is needed (Nilsson 1992, Pettersson & Nilsson 1993). In other words, the plant is not supposed to be dependent on repeated pollinator visits for reproductive success.

1.3 What is the role of local adaptation in a root hemiparasite-host interaction in small populations of *Euphrasia rostkoviana* (IV)

Local adaptation is usually indicated as a higher fitness of genotypes in their site of origin (Dudley 1996). The most important conditions necessary for local adaptation to develop are localized selection and limited gene flow (e.g. Waser & Price 1989, Bennington & McGraw 1995). Some plants can experience natural selection on a very fine spatial scale due to their sessile habit and restricted pollen and seed dispersal (Schmitt & Gamble 1990). Studies in plants have demonstrated local adaptation to abiotic conditions (e.g. Hickey & McNeilly 1975, Wang et al. 1997), biotic conditions (e.g. Turkington & Harper 1979, Schoen et al. 1986) or both (e.g. Schemske 1984, Waser & Price 1985, Emms & Arnold 1997).

Local adaptation could also have a role in the co-evolutionary relationship between parasitic plants and their hosts. Both the holoparasitic stem parasites and the hemiparasitic root parasites possess haustoria, special contact organs by which they rob water, mineral nutrients and even carbohydrates from the vascular system of their hosts (e.g. Press et al. 1993). Therefore, the host species may show strong resistance or tolerance to parasites. It can be predicted that the hemiparasites may be locally adapted to their hosts, i.e., to the biotic environment, and that the hemiparasites may have a "competitive" advantage over their hosts. In contrast, at a certain time, hosts may have a "competitive" advantage over the hemiparasites as an outcome of the antagonistic, co-evolutionary arms race between the two species (Dawkins & Krebs 1979, Gandon et al. 1996, Morand et al. 1996). The performance of individuals and population viability of the endangered hemiparasite *E. rostkoviana* could therefore be affected by the level of local adaptation in the hemiparasite-host interaction. In principle, poorly adapted populations of the annual *E. rostkoviana* face a danger of extinction within a few years if seed production fails and if the seed bank is limited.

1.4 Is there an association among genetic diversity, population size and fitness in small populations of *Lychnis viscaria*? (V)

Genetic diversity, the evolutionary potential for the future, is considered especially important for rare species, such as *L. viscaria*, that usually has small and isolated populations. Genetic diversity is usually low in isolated populations as a consequence of genetic drift, inbreeding, bottlenecks, and founder effects (e.g. Holsinger 1993, Fenster & Dudash 1994, Hoffman & Blows 1994). These factors have a large impact on differentiation among populations. Thus, species conservation should be based on information of the overall level of genetic diversity and its distribution within and among populations. Besides this basic information, species conservation should focus on differences between geographically peripheral and central populations.

Peripheral populations have several characters in common: they are small, isolated, and occur in ecologically marginal habitats (e.g. Lawton 1993, Lesica & Allendorf 1995). In several cases this has resulted in reduced genetic diversity (Lesica & Allendorf 1992, 1995, Frankham 1996), but this is not always the case (Ellstrand & Elam 1993). Thus, the conservation value of these populations has been questioned. On the other hand, it has been suggested that stronger selection pressures favoring heterozygosity in peripheral populations could even increase the level of genetic diversity in those populations and that they could be a source of future speciation events and local adaptations having great conservation value (reviewed in Lesica & Allendorf 1992, 1995).

At the moment, the combined importance of genetic diversity, population size, and geographic location of populations for fitness components of individuals is unclear (e.g. Schemske et al. 1994, Vrijenhoek 1994, Fischer & Matthies 1998). However, fitness components, such as germination rate, seedling growth rate, and seed production, which are subjected to selection, may be crucial for the persistence of small and isolated plant populations (e.g. Menges 1991). Thus, the viability of individuals could be used as one criteria in setting conservation priorities (Holsinger & Godtlieb 1991, Hedrick & Savolainen 1996).

1.5 The aims of this study

In this thesis I attempt to demonstrate some of the most important ecological and genetic factors affecting reproductive success and further population viability in small populations of three rare or endangered vascular plant species. More specifically, in studies focusing on *D. incarnata*, I aim to find out how nectar-containing and otherwise attractive plants flowering simultaneously in the habitat will affect the fruit set of the orchid (I), how the attractiveness of an individual orchid affects its male and female reproductive success (II), and how population size, location and their combination affect male and female reproductive success (II). I examine the validity of two hypotheses explaining the evolution of deceptive pollination by performing two hand-pollination experiments (III). Furthermore, I examine the role of local adaptation and co-evolution in a hemiparasite-host relationship in small populations of *E. rostkoviana* by studying growth and survival of hemiparasites grown together with familiar or unfamiliar hosts (IV). Finally, I study the associations among genetic diversity, population size and fitness in a network of isolated populations of *L. viscaria* (V).

2 MATERIALS AND METHODS

2.1 Study species (I-V)

Dactylorhiza incarnata (L.) Soó (Orchidaceae) is a perennial orchid that has a disjunct distribution in Northern Europe extending up to the 70° latitude (Hultén 1971). In Finland it is classified as an endangered species, often occurring in small and patchy populations (Rassi et al. 1992). Each plant produces on average fifteen bright pink nectarless (Nilsson 1981, Lammi & Kuitunen 1995) and scentless (Nilsson 1980, 1981) flowers, borne on a spike. Each flower has two pollinia. The species is self-compatible, but spontaneous autogamy is absent or very rare in nature. The species is pollinator limited within a season, because after hand-pollination fruit set may rise up to 100 % (Lammi & Kuitunen 1995). In pollination *D. incarnata* exploits short visits by inexperienced or unconditioned bumble bee workers and attractive floral display is needed to achieve a good pollination success. Deception occurs throughout anthesis, because fresh bumble bee workers emerge from their nests during the whole summer (Nilsson 1980). Fruit set is typically 20-50 % in natural populations (Lammi & Kuitunen 1995).

Euphrasia rostkoviana Hayne subsp. *fennica* (Kihlman) Karlsson (Scrophulariaceae) is an annual root hemiparasite and it has a disjunct distribution in Northern Europe extending up to the 65° latitude (Hultén 1971). In Finland *E. rostkoviana* is an endangered species occurring mostly on dry meadows and semi-natural grasslands. With the abandonment of traditional methods of agriculture, the species has declined drastically during the last decades (Rassi et al. 1992). The species is diploid, sexually reproducing (not apomictic) and self-compatible. The flowers are entomophilous with low rates of outcrossing and overall low pollinator visitation rates in nature (Yeo 1966, 1968). Hermaphroditic and protogynous flowers are pollinated by hover-flies (Diptera, Syrphidae) and bees (Hymenoptera, Apidae, s.l.). The genus produces zero to sixteen seeds per capsule (Yeo 1966) and for *E. rostkoviana* germination percentages up to 40 % have been recorded (Yeo 1964).

Lychnis viscaria (Bernh.) (Caryophyllaceae) is a perennial herb found in most parts of Central Europe, and its distribution in Northern Europe extends to the 62° latitude (Hultén 1971, Wilson et al. 1995). *L. viscaria* was much more common a century ago, when agriculture constantly created new dry meadows suitable for the species. In the province of Central Finland the species is listed as regionally endangered, but it is not threatened nationally. The number of inflorescences per plant ranges from 1-50 with about 20-25 flowers in each inflorescence. The species is usually self-compatible, but partial self-incompatibility occurs in some populations (Wilson et al. 1995). Despite protandry self-pollination occurs, but seed set is low after selfing (Jennersten et al. 1988). Flowers are visited by a wide range of mostly diurnal insects, among which bumble bees and butterflies are most frequent (Jennersten 1988). Seeds germinate readily (over 90%) without any pretreatments in the autumn as well as in the spring and there is no evidence for dormancy (Wilson et al. 1995).

2.2 Study areas (I-V)

The studies focusing on reproductive success of *D. incarnata* were carried out at several mires situated in Central Finland. The experiment described in paper (I) was undertaken during 1990-1992 at the Katajaneva mire, situated in Toivakka, Central Finland (62°30'N, 26°12'E). The data presented in paper (II) was gathered from 16 mires situated in Central Finland in the municipalities of Toivakka, Leivonmäki, Pihtipudas, Viitasaari, and Kinnula during 1992-1993. The hand-pollination experiments described in paper (III) were carried out at Katajaneva mire in 1994 and at Paanasenneva mire situated in Pihtipudas, Central Finland (63°31'N, 25°32'E) in 1993. The habitats of *D. incarnata* within the mires were mesotrophic sedge fens or *Sphagnum papillosum* fens.

Seeds of *E. rostkoviana* were collected from six populations in central Finland at the municipalities of Äetsä (Kiimasjärvi east and west, 61°18'N, 22°41'E), Viljakkala (Lopenkulma, 61°46'N, 23°29'E), Nokia (Maatialanharju, 61°29'N, 23°29'E) and Jyväskylä (Aholaita east and west, 62°16'N, 25°48'E). The number of plants varied between 30 and 100 per population. The *Euphrasia* populations were restricted to very small (100-5000 m²) patches within dry meadows. The experiment was carried out at the facilities of Department of Biological and Environmental Science, University of Jyväskylä.

Genetics and ecology of *L. viscaria* were studied in both peripheral and central populations within its distribution area in Finland. In the surroundings of Jyväskylä (62°15'N, 25°45'E), *L. viscaria* occurs in its northern range at rather small and isolated patches in rocky cliffs (hereafter peripheral populations). In the surroundings of Tampere (61°30'N, 23°45'E), situated at the main distribution area, 150 km SW of Jyväskylä (hereafter central populations), the species occurs in larger populations mainly on roadsides and dry meadows. Some of the ecological measurements were carried out at facilities of the Department of Biological and Environmental Science, University of Jyväskylä. Genetic analyses were carried out at the Konnevesi Research Station.

2.3 Methods to study reproductive success in *D. incarnata* (I-III)

In order to test the "magnet-species theory" (I) three separate study-areas were formed inside the main population of *D. incarnata* at the Katajaneva mire, each having similar natural density of the orchids (0.4 plants / m²). These areas were about 80 m apart from each other in a triangular pattern. Purple-red garden violets (*Viola x wittrockiana*) were added to the first area (these were approximately the same colour as *D. incarnata*), light blue violets (*Viola x wittrockiana*) were added to the second area and the third area served as a control area without any addition. Violets of different colours were used to test whether pollinators use colours as a cue to choose their food flowers. During this three-year long experiment (1990-1992) the study areas received a different treatment each year. The densities of violets were similar in both areas (0.5 plants / m²) each year. The violets, each having approximately five open flowers, were placed in plastic-boxes near each orchid in the experiment just before mid June, when the blossoming of *D. incarnata* began. Altogether, each study-area contained about 25 violets each year and most of the orchids in the study areas were included in the experiment. Percentage fruit set was used as a measure of reproductive success. In papers I-III we used percentage of fruit set or pollen removed per plant rather than absolute values, because in this way the long-term costs of reproduction in the perennial orchid are included more accurately to the study.

To study the importance of plant attractiveness for pollination success (II) the length of inflorescence, the maximum height of individual plants, and the number of flowers per plant were measured. Because these variables were highly autocorrelated, the first principal component on the above mentioned variables was used as an indicator of attractiveness in most of the statistical tests. Male reproductive success was determined as the percentage of pollinia removed per individual, and female reproductive success as the percentage of fruits produced. Fruit set was measured from six to eight weeks after flowering, at a time the ovaries were fully swollen. The ratio between overall male and female reproductive success of plants in a population, the equivalence factor (E), was determined by the formula of Lloyd (1980).

To study the effect of population detectability on reproductive success (II) the distance from the centre of the orchid populations to the nearest forested area with nectar-containing species present was measured from topographic maps (1:20 000). The counted number of flowering plants per population was used as a measure of population size.

The two hypotheses concerning the evolution of deceptive pollination were studied experimentally (III). In the first hand-pollination experiment, testing the "outcrossing" hypothesis and hybridization, the experimental flowers were pollinated with an equal amount of pollen (two pollinia) from different sources. The treatments were: autogamy, outcrossing (20 m) and outcrossing (16 km). We used the pollinia from the closely related *Dactylorhiza maculata* as a fourth treatment. All the four pollination treatments were performed within an individual plant. In each recipient inflorescence, 2-3 random flowers were pollinated with pollinia from the same source.

In the second hand-pollination experiment, testing the "pollinia" hypothesis, we studied the effect of additional pollinia received per plant. Results of the same experimental treatment were gained from the first experiment as well. We also studied the effect of number of pollinia received per stigma (a half, 2 or 3 pollinia) on reproductive success. We took pollinia from *D. incarnata* individuals five meters away from the experimental plants. In this experiment, we performed only one type of treatment for each experimental plant. In each inflorescence, we pollinated 4-11 random flowers with the same number of pollinia. The treatments were randomized over maternal plants of different size.

In both experiments about 60 % of the flowers on each plant were hand-pollinated to elicit reproductive effort on the maternal plants equally. We covered the inflorescences with nylon bags to exclude natural pollinators. We used a toothpick to withdraw a pollinium and to place it on the stigma of an experimental flower marked individually. In the first experiment we estimated female reproductive success as: 1. the percentage of fruit set, 2. the seed set per fruit and 3. the percentage of seeds with well-developed embryos. In the second experiment, the same variables except seed set were studied.

2.4 Local adaptation experiment (IV)

I studied experimentally the role of local adaptation in a hemiparasite-host relationship in five populations of *E. rostkoviana*. I planted *E. rostkoviana* seedlings together with *Agrostis capillaris* (L.) hosts originating from the centre of the same *Euphrasia* population (hereafter familiar hosts) or with hosts originating from a distance of approximately 80 meters (hereafter unfamiliar hosts). Both hemiparasite and host seedlings added to the same pots were grown from seeds germinated indoors during the early spring. To measure the growth of hemiparasites grown together with familiar or unfamiliar hosts their leaf number and maximum height was censused one, two and three months after transplanting into the pots. At the same time, the stalk number of hosts was counted. After two and three months the number of flowers produced by hemiparasites was also counted. At the end of the experiment, I weighed the dry biomass of the hemiparasites and the above and below ground biomass of hosts. In the same experiment, the survival of individual hemiparasites grown together with familiar or unfamiliar hosts was counted in five populations as the total number of hemiparasites transplanted per treatment minus the number of hemiparasites that died.

2.5 Genetic diversity, population size and fitness (V)

The 11 study populations of *L. viscaria* included in the electrophoresis consisted of 7-1000 flowering individuals per population. Seed capsules were collected randomly and several seedlings per maternal plant were grown under standard

greenhouse conditions. The amount of isozyme variation of seedlings was determined by means of horizontal starch gel electrophoresis as described in Wendel & Weeden (1989) and May (1992). Three gel and electrode buffer systems were used to resolve the isozymes, and a total of 13 enzymes and 17 loci were screened. Six loci (F-est-1, F-est-2, Gpi-2, Pgm-2, Skd-1, Sod-1) were polymorphic. Genetic diversity for each population was assessed by Nei's (1978) unbiased estimate of expected heterozygosity (H_{exp}), observed heterozygosity (H_{obs}), percentage of polymorphic loci (P) and mean number of alleles per locus (A). Population structure and inbreeding coefficients were calculated by F-statistics (Wright 1921,1969) according to the protocol of Weir & Cockerham (1984). The F-statistics were used to indirectly estimate gene flow and the migration rate (N_{em}) between populations. The migration rate was estimated as: $N_{em} = (1/F_{ST} - 1)/4$ (Slatkin & Barton 1989). Associations between Nei's (1972) unbiased genetic distance and linear geographic distance were estimated using Mantel test. To illustrate mean genetic distances among populations a UPGMA phenogram was constructed.

For the ecological studies we sampled four additional central populations. To determine germination percentage, 50 fully developed seeds per naturally pollinated maternal plant were germinated in petri dishes. After three weeks the final germination percentage was scored. To determine the seedling growth rate, seedlings were grown under standard greenhouse conditions. After six weeks we assessed the above-ground dry biomass of seedlings. To estimate total seed yield per fruit, we first counted the number of seeds per one random fruit and then multiplied total seed number per fruit by the mean mass of one seed.

2.6 Statistical analyses

Statistical tests were performed with SPSS for Windows. Non-parametric tests were applied when the assumptions of parametric tests were not met. Population genetic statistics were mostly calculated using the BIOSYS-1 program. Statistical tests are described in more details in papers I-V.

3 RESULTS AND DISCUSSION

3.1 Reproductive success of *D. incarnata*

3.1.1 The "magnet-species theory" (I)

On average, the percentage fruit set of *D. incarnata* did not differ between the three study years. However, there were differences in fruit set between the areas where *D. incarnata* was growing together with *Viola x wittrockiana* or alone. The fruit set of *D. incarnata* was greater if growing alone than the fruit set of *D. incarnata* growing with *Viola x wittrockiana*. The fruit set of *D. incarnata* was especially low when growing with purple red *Viola x wittrockiana* which was approximately of the same colour as the orchids.

The results support the hypothesis of Nilsson (1981), who stated that the deceptive pollination system of marsh dactylorchids functions best in remote habitats like marshes where there are no concurrently flowering plants attractive to the pollinators. In this study, the addition of violets may have led to shorter flight-distances of the pollinators. However, pollinators remaining in areas where nectar-containing species were added did not benefit the orchids. In these areas bumble bees probably visited violets at the expense of the orchids' pollination success. In this experiment it appeared that interspecific exploitation competition, where one species draws pollinators away from another species (Pleasants 1980, Zimmerman 1980), took place in favour of the violets at the expense of the orchids. As a consequence, the addition of the violets produced no evidence for the "magnet-species theory" (e.g. Thomson 1978).

3.1.2 The importance of attractive floral display, population size and population detectability (II)

In 4 out of 16 populations attractive plants (PC1) donated more pollinia than less attractive plants. Attractiveness (PC1) of the individual orchids did not increase female reproductive success in any of the populations. However, higher

standard deviation in the components of attractiveness within a population had a positive effect on pollinia removal most likely because pollinators' ability to develop avoidance learning towards the deceptive orchids was made more difficult (e.g. Nilsson 1980, Cohen & Shmida 1993). At the population level, the size of populations did not increase fruit set or pollinia removal. This result agrees with other studies of nectarless orchids, (e.g. Fritz & Nilsson 1994) but is controversial to most plant species with pollinator rewards (e.g. Rathcke & Jules 1993, Olesen & Jain 1994). The distance between orchid populations and the nearest forested areas with food plants for the pollinators did not affect fruit set or pollinia removal. Thus, even the most distant populations were found by the pollinators. Pollinia removal was strongly affected by year, although fruit set was not affected by year.

The equivalence factor (E) was associated with the mean attractiveness of the populations. Those populations including on an average more attractive individuals had lower E values, meaning that more pollinia were removed for each fruit than was set. The equivalence factor of populations was most strongly affected by year. Population size and distance were not associated with E. From the evidence presented here it is obvious that variation especially between years and also between populations in male and female reproductive success may be extensive (e.g. Alexandersson & Ågren 1996).

3.1.3 The evolution of deceptive pollination (III)

The first experiment tested the "outcrossing" hypothesis and the effect of hybridization. Pollinia source did not affect percentage fruit set, seed set or percentage of seeds with well-developed embryos.

In the first experiment the plants were able to mature up to 60 % of their total flower production in each treatment, indicating pollinator limitation compared to the normal 20-50 % fruit set in natural populations. However, in the second experiment the plants which were hand-pollinated with two pollinia per flower could mature only about 30 % of their total flower production.

In the second experiment the number of pollinia per stigma affected the proportion of flowers setting fruit and percentage of seeds with well developed embryos. Increasing the number of pollinia used per stigma had a positive effect on reproductive success. The significant results in percentage fruit set and percentage of seeds with well-developed embryos were due to differences between treatments using a half pollinium and three pollinia. There were no differences in reproductive success between treatments using a half pollinium and two pollinia or between two and three pollinia used.

Avoidance of selfing is commonly accepted as an explanation for the evolution of deceptive pollination (Nilsson 1992). However, our results of the early phases of the orchids' life cycle did not seem to clearly support the idea, because outcrossing did not lead to a higher reproductive success compared to selfing. Based on the results, we were unable to detect inbreeding depression within and outbreeding depression within or between populations in relation to crossing distances. Our results indicate, that populations may be highly isolated, inbreeding and purging may be possible and therefore selfing and inbreeding may not reduce female reproductive success. The possibilities for outbreed-

ding depression are also ignored in this hypothesis, because it sets no limits to the maximal outcrossing distances after which reproductive success will be lower (e.g. Waser & Price 1983, 1993). Evidently the "outcrossing" hypothesis may have limited application, because genetic structure may vary markedly among populations.

Based on our results, hybridization may commonly occur between *D. incarnata* and *D. maculata*. Consequently, increased crossing distance could be associated with hybridization which could counteract the assumed benefit of increased crossing distance (e.g. Nilsson 1992). The harmful consequences of hybridization include demographic effects, genetic assimilation of a rare taxon by a numerically larger one, loss of locally adapted populations and outbreeding depression (e.g. Rieseberg 1991, Ellstrand & Elam 1993, Levin et al. 1996). The benefit of hybridization could come from increased viability after heterosis and adaptation to new environments (e.g. Rieseberg 1991, Waser 1993, Levin et al. 1996). Our separate experiments indicated that the number of pollinia had a positive effect on female reproductive success, and interspecific pollinia produced seeds as well. This suggests, that genetic assimilation between species could be common and effective in the mixed populations of *D. incarnata* and *D. maculata*.

Our results indicated that repeated pollinator visits should give higher total reproductive success and, in fact, several orchids are pollinator limited. However, as shown by the two experiments, the extent of pollinator limitation may vary between populations. We assume that the "pollinia" hypothesis could only be verified by a long-term study monitoring the fitness of maternal plants and their progeny (Waser & Price 1994), but this is very difficult to accomplish in orchids.

3.2 Local adaptation in a root hemiparasite-host interaction (IV)

After one month of growth, the height and the number of leaves of *E. rostkoviana* hemiparasites were not affected by the origin of their hosts. The differences in growth were due to between population effects. The situation remained constant after three months. Hemiparasite biomass was not affected by the origin of the hosts. The percentage of hemiparasites surviving after one, two and three months was not affected by the origin of the hosts although there was a weak tendency towards better survival of hemiparasites with familiar hosts than with unfamiliar hosts. All variables used to measure hemiparasite performance during its complete life cycle gave only limited support for the local adaptation hypothesis.

The annual life cycle should have allowed detection of local adaptation and co-adaptive interactions over the duration of the hemiparasitic life-time. In this study, the only indication of local adaptation was the increased hemiparasitic survival when attached to familiar hosts after the first, second and third month. However, there may have been some co-evolutionary interactions between parasites and hosts, because the familiar hosts suffered less from pa-

rasitism as indicated by their higher biomass. Thus, the familiar hosts had some competitive advantage or tolerance over their hemiparasites, although the advantage was not strong enough to prevent hemiparasite growth.

There are several possible reasons why the hemiparasites seemed not to be locally adapted to their hosts. In general, there is always a dynamic relationship between parasites and hosts where parasites track host allele frequencies with a lag (Morand et al. 1996, Khibnik & Kondrashov 1997). Firstly, one possible reason could be the commonly occurring hybridization in *Euphrasia* which may prevent local adaptations from developing (Levin et al. 1996). Secondly, 30 host species have been recorded in total for the genus *Euphrasia* and several suitable host species may exist for one single population (Wilkins 1963, Yeo 1964). This may reduce the parasite-host co-evolutionary competition and cause disruptive selection pressures preventing the hemiparasites from becoming locally adapted to their hosts. However, studies focusing exclusively on separate populations may not be sufficient to judge the final role of local adaptation, because demographic stochasticity in a metapopulation may affect the relationship between parasites and hosts (Wilson & Hassell 1997).

3.3 Association among genetic diversity, population size and fitness in *L. viscaria* (V)

Our results showed that the level of genetic diversity varied substantially among populations ($H_{\text{exp}} = 0.000 - 0.116$) and the total level of genetic diversity (mean $H_{\text{exp}} = 0.056$) was low compared to other species with similar life-history attributes. The peripheral populations of *L. viscaria* had less genetic variation (mean $H_{\text{exp}} = 0.034$) than the central ones (0.114). Analysis of genetic structure suggested limited gene flow (mean $F_{\text{ST}} = 0.430$) and high differentiation among populations emphasizing the role of genetic drift ($N_e m = 0.33$). Isolation was even higher than expected from the physical distance among populations. No significant associations between geographic distances and genetic distances were found (Mantel test, $z = 0.93$, $p = 0.176$, 56 comparisons). Genetic distances were not associated with the geographic distribution of populations.

The low level of genetic variation in *L. viscaria* is possibly a result of one or several global bottlenecks in connection with repeated extinctions and recolonizations resulting from Quaternary glaciations (Haraldsen & Wesenberg 1993). The level of genetic differentiation among *L. viscaria* populations is high compared to 115 species reviewed by Govindaraju (1988). Thus, our results in *L. viscaria* strongly suggest that populations are influenced by genetic drift independently of each other and of geographic distance or area. Given limited pollen and seed dispersal mechanisms of *L. viscaria* (Wilson et al. 1995) and the long geographic distance among populations, differentiation was expected. Our results did not support the hypothesis that peripheral populations should have more genetic variation due to stronger selection pressure (Lesica & Allendorf 1992, 1995).

Additionally, we focused on the association between population size and genetic diversity and possible fitness impacts of these factors. Population size was positively correlated with genetic diversity. Population size and genetic diversity, however, were not associated with fitness components such as germination rate, seedling mass or seed yield. There were no differences in the measured fitness components between peripheral and central populations.

In *L. viscaria* the smaller populations had less genetic variation presumably because of genetic drift, founder effects, and bottlenecks (e.g. Frankham 1996, Godt et al. 1996, Siikamäki & Lammi 1998). The mating system and pollination success of *L. viscaria* may also have had some impact, although they are difficult to separate from the other factors mentioned above. In general, the results concerning association between population characters and individual fitness seem to vary according to species (e.g. Vrijenhoek 1994).

In this study, there were no differences in the fitness components between peripheral and central populations. This is a positive result for conservation biology, because quite often small and peripheral populations are considered less viable than central and large populations (e.g. Lesica & Allendorf 1992, 1995). Based on the results we would strongly recommend the conservation of small, isolated and peripheral populations as well. It seems to us, that the conservation value of these populations is often underestimated, especially if the lack of genetic diversity has been observed. These populations may be quite viable without difficulties e.g. in reproduction, germination and seedling establishment (Van Treuren et al. 1993, Widen 1993, Hauser & Loeschcke 1994, Ouborg & Van Treuren 1995). If we look at the issue from a longer time perspective, the importance of isolated and peripheral populations is emphasized, provided that there is some genetic variation left (Sun 1996, Young et al. 1996). In these habitats, if in any, different regimes of natural selection are most likely to affect gene frequencies and unique genotypes may be formed and selected for.

4 CONCLUSIONS

In this thesis I have attempted to demonstrate some of the most important ecological and genetic factors affecting plant reproductive success and population viability by studying three rare or endangered vascular plant species occurring in small and isolated populations. In the studies focusing on reproductive success (I - III), I found that deceptive pollination system of *D. incarnata* functioned best in remote habitats like mires where there are no concurrently flowering plants attractive to the pollinators. However, individual (e.g. floral display) and other population characteristics (e.g. size and detectability) seemed to have only secondary importance for male and female reproductive success. Based on the early phases of the species' life cycle, the abundance of pollinators and the amount of pollinia received was found to be more important for the reproductive success of *D. incarnata* than the genetic quality and the origin of pollinia.

These results could give a new prospect to understand the evolution of deceptive pollination and reproductive success in orchids. In the Finnish orchid-flora, 11 out of 34 species (32%) offer no nectar for their pollinators, yet they are pollinated by insects and totally rely on sexual reproduction. At the moment the proportion of endangered species in the family Orchidaceae (52,9%) is one of the highest in the Finnish flora (Rassi et al. 1992, Lammi 1997). Thus, these sophisticated pollination systems may have some consequences for population persistence through varying pollination success. In fact, it would be useful to recognize temporal and spatial fluidity in plant-pollinator interactions and to notice that selection and gene flow vary in time and space. Indeed, some workers have recently taken a healthily sceptical approach to the classical pollination syndromes (Waser et al. 1996, Ollerton 1998). This approach may gain new insight to the understanding of reproductive success in nectarless species. Hopefully, these conclusions may be applied to nectar-containing species as well.

Other ecological-genetic factors, such as local adaptation, may also have an important role for ensuring population persistence. In the *Euphrasia* study (IV), all variables used to measure hemiparasite performance during its complete life cycle gave only limited support for local adaptation. In terms of conserva-

tion biology, the lack of local adaptation is a positive occurrence. There seems to be unlimited potential for *Euphrasia* reintroductions into suitable habitats and possibly to transplants among populations due to the lack of specific host requirements. However, this result is based on examining one host species only. If there had been strong indications of local adaptations within each population, transplanting and reintroductions should be ruled out as management tools, because of outbreeding depression and due to the lack of suitable hosts.

The results of the isolated and small populations of *L. viscaria* showed that the level of genetic diversity varied substantially among populations and in general it was low (V). The peripheral populations of *L. viscaria* had less genetic variation than the central ones. Population size was positively correlated with genetic diversity. Population size and genetic diversity, however, were not associated with fitness components. Even though small and peripheral populations had lower level of genetic variation, they were as viable as larger populations and did not show any signs of reduced fitness. Based on the results I would recommend the conservation of small, isolated and peripheral vascular plant populations as well. If we look at the issue from a longer time perspective, the importance of isolated and peripheral populations is enhanced, provided that there is some genetic variation left. In these populations unique genotypes may be selected for as local adaptation proceeds. Thus, the conservation value of these populations should not be underestimated.

Clearly, one thesis is not enough to resolve all the problems related to endangered plants and their conservation. However, I believe that this thesis has shown how important it is to combine both ecological and genetic approaches in order to achieve a broad understanding of species' future. One possibility for me would have been to study reproductive success, local adaptation and genetics in one species only. However, by choosing different species, but in several ways facing the same problems for survival, I wanted to underline the importance of grouping species into larger units. Indeed, we would need several genetic and ecological projects working in close co-operation with other experts of their own field. In such a network of projects, each focusing on different group of plant species representing different life-history strategies, tactics and traits, results could be achieved within a proper time scale to prevent at least some of the extinctions ahead of us. As an outcome of such projects, managing suggestions could be given to different groups of species, based on their life-history strategies. Within these groups of species, I believe, some general conclusions may emerge, and management recommendations could be given to a larger number of species.

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YHTEENVETO

Pienten kasvipopulaatioiden lisääntymismenestys, paikallinen sopeutuminen ja geneettinen monimuotoisuus

Tutkin väitöskirjatyössäni ekologisten tekijöiden sekä geneettisen muuntelun määrän merkitystä pienten ja muista erillään olevien (isoloituneiden) kasvipopulaatioiden säilymiselle. Alan tutkijat ovat laajalti yksimielisiä siitä, että juuri nämä tekijät, sattuman lisäksi, vaikuttavat eniten kasvipopulaatioiden haviämiin tai säilymiseen. Erityisesti keskityn selvittämään lisääntymismenestystä, paikallisia sopeumia ja geneettisen muuntelun määrää esiintymissä. Osatutkimusteni kohteiksi valitsin punakämmekän (*Dactylorhiza incarnata*), ahosilmäruohon (*Euphrasia rostkoviana*) ja mäkitervakon (*Lychnis viscaria*), joiden kaikkien lisääntymismenestys riippuu pölyttäjähönteisistä. Tutkimusalueillani Keski-Suomessa kaikki lajit esiintyvät pienissä ja isoloituneissa populaatioissa kaukana toisista saman lajin esiintymistä.

Väitöskirjatyöni osatutkimuksissa I-III keskityn erityisesti lisääntymismenestykseen vaikuttavien tekijöiden selvittämiseen uhanalaisella punakämmekällä. Punakämmekä on monivuotinen, erityisesti ravinteikkaiden nevojen ja rämeiden orkidea. Toisin kuin useimmat hyönteispölytteiset kasvit, punakämmekä ei tuota mettä, mutta silti sen pölytysmenestys on kimalaisten varassa. Lajin pölytys perustuu kimalaisten petteämiseen korealla ulkonäöllä. Nuoret ja vastakuoriutuneet kimalaistyöläiset, joita syntyy läpi kesän, ovat helpoiten petettävissä.

Pölytysmenestykseen vaikuttavat olosuhteet ovat kuitenkin olleet jonkin verran epäselviä, ja jopa ristiriitaisia hypoteesejä on esitetty. Ensimmäisessä väitöskirjani osatutkimuksessa selvitin, vaikuttaako medellisten kasvilajien läsnäolo punakämmekän kasvupaikalla kämmekän lisääntymismenestykseen. Lajin on väitetty sekä hyötyvän että kärsivän siitä, että yleensä sen kasvupaikoilla ei samanaikaisesti esiinny lainkaan kukkivia medellisiä kasvilajeja. Tutkimuksessa lisäsin suolle medellisiä, purkkeihin istutettuja puutarha-orvokkeja. Kunakin kolmena tutkimusvuotena kahteen esiintymään lisättiin orvokkeja ja

yksi esiintymä toimi kontrollialueena. Tulokset osoittivat selkeästi, että punakämmekän lisääntymismenestys oli parempi, kun esiintymiin ei ollut lisätty medellisiä lajeja. Kun medellisiä kasveja oli tarjolla, pölyttäjät valitsivat ne kämmeköiden kustannuksella. Luonnonvalinta on ilmeisesti johtanut siihen, että punakämmekän esiintymät sijaitsivat juuri avosoilla, joissa ei tavata medellisiä kasvilajeja samanaikaisesti.

Toisessa osatutkimuksessa selvitin, mikä on suurikokoisen ja houkuttelevan kukinnan merkitys yksilön lisääntymismenestykselle. Näköaistinsa varassa toimivat pölyttäjät olettavat yleensä saavansa suurista kukinnoista enemmän mettä ja hakeutuvat siten aktiivisesti suurimpiin kukintoihin. Punakämmekän medettömyys saattaisi vielä vahvistaa ilmiötä, koska suuri kukinto on punakämmekän ainoa keino houkuttaa pölyttäjiä. Tulokset osoittivat, että siitepölyn vienti ja siemenkotien tuotanto eivät lisääntyneet yksilön houkuttelevuuden kasvaessa. Sensijaan suuri populaation sisäinen hajonta yksilön houkuttelevuudessa saattaa todennäköisesti lisätä yksilöiden keskimääräistä lisääntymismenestystä, koska pölyttäjän on vaikeampaa oppia tunnistamaan petollista kasvilajia sen kukinnan koon vaihdellessa.

Samassa osatutkimuksessa selvitin myös punakämmekän populaatiokoon merkitystä yksilöiden lisääntymismenestykselle. Yleensä medellisillä lajeilla suuri populaatiokoko johtaa parempaan lisääntymismenestykseen, koska suuret kasviryhmät vetävät paremmin pölyttäjiä puoleensa. On kuitenkin esitetty, että medettömät lajit menestyisivät paremmin pienissä populaatioissa, joissa pölyttäjät eivät yhtä hyvin opi tunnistamaan medetöntä pettäjäkasvia. Tulokseni 16:sta populaatiosta osoittivat kuitenkin, että siitepölyn luovutus ja siemenkotatuotanto eivät riippuneet populaatiokoosta. Myöskään kämmekäesiintymän etäisyys suon reunasta, jossa pölyttäjien todelliset ravintokasvit kasvavat, ei vaikuttanut punakämmekän lisääntymismenestykseen. Pölyttäjät löytävät siis suurtenkin nevojen keskellä olevat punakämmekä-esiintymät.

Väitöskirjatyöni kolmannessa osatutkimuksessa selvitin pölytyskokein punakämmekän petospölytyssysteemin evolutiivista kehitystä. Nykyisten medettömien lajien kantamuodot ovat miljoonia vuosia sitten olleet medellisiä. Medettömyyden synnystä on esitetty useita hypoteesejä, jotka kaikki lähtevät siitä, että medettömyydellä pitäisi saavuttaa jotain muuta hyötyä, koska pölyttäjiä ei kyetä enää houkuttelemaan yhtä paljon kuin meden kanssa.

Yhtenä vaihtoehtona on esitetty, että pölyttäjän lentomatka seuraavaan kasvivyksilöön pitenisi sen vierailtua medettömässä kasvissa. Tästä oletetaan olevan hyötyä kasville, koska todennäköisyys saada vierasta, elinvoimaista siitepölyä kasvaksi lentomatkan pidetessä. Samalla yksilö välttäisi pariutumisen lähellään kasvavan, todennäköisesti oman lähisukulaisensa kanssa. Tutkin hypoteesia tekemällä käsipölytyksiä, joissa siirrettiin punakämmekän siitepölymyhkyjä eri etäisyyksiltä koekasvien kukkien eminluoteille ja mitattiin syntyvien alkuiden ja siemenkotien määrä. Lisääntymismenestyksessä ei ollut havaittavissa eroja käytettäessä yksilön omaa, lähiyksilöiden tai toiselta suolta tuotua siitepölyä. Miltei samoilla kasvupaikoilla kasvavien maariankämmeköiden siitepöly johti sekin hyvään lisääntymismenestykseen ja vähälukuisempaa punakämmekää saattaakin uhata risteytymisen kautta tapahtuva sulautuminen runsaampaan sukulaislajiinsa. Risteytyminen saattaa jopa lisääntyä, mikäli pö-

lyttäjäien lentomatkat pitenevät ulottumaan maariankämmeköiden kasvupaikoille asti. Näin ollen, ristipölytyksen edullisuus ei vaikuttanut uskottavalta selitykseltä petospölytyksen evolutiiviselle kehitykselle.

Toinen hypoteesi, jolla petospölytyksen evoluutiota on selitetty, perustuu lajin tiiviisiin siitepölymyhkyihin, joissa kaikissa on suuri määrä siitepölyhiukkasia. Usein kokonainen myhky siirtyy pölyttäjän toimesta toiseen kasviyksilöön. Onkin esitetty, että lajista on kehittynyt medetön, koska sille ei koidu haittaa, vaikka vain muutamaa pölyttäjää onnistuttaisiin pettämään. Jo parinkin pölyttäjän tuomat siitepölymyhkyt tuottavat runsaan siemensadon ja takaavat jälkeläistuotannon. Käsi-pölytyksissä lisäksi sekä koko kasvin että yksittäisen kukan saamaa siitepölymäärää, jolloin siementuotto parani. Siitepölymyhkyjen olemassaolo ei siis vaikuttanut uskottavalta selitykseltä petoksen evoluutiolle. Ilmiötä selittävät todennäköisemmin lisääntymismenestyksen ja resurssien tasapainottamiseen perustuvat hypoteesit.

Yhteenvetona punakämmekätöistä voi todeta, että tutkimukset osoittivat pölyttäjien saatavuuden ja siitepölyn määrän merkityksen olevan tärkeämpää lajin lisääntymismenestykselle kuin saadun siitepölyn laadun ja alkuperän. Tietoa voidaan hyödyntää punakämmekän ja eräiltä osin muidenkin maamme lukuisten medettömien kämmeköiden suojelutyössä.

Väitöskirjani neljänessä osatutkimuksessa tutkin paikallisten sopeumien merkitystä puoliloiskasvin (ahosilmäruoho) ja sen isäntäkasvin (nurmirölli) välisessä vuorovaikutussuhteessa. Paikallisella sopeumalla tarkoitetaan yleisesti ottaen tietyn populaation yksilöille luonnonvalinnan seurauksena syntyneitä ominaisuuksia, joiden ansiosta yksilöt menestyvät elinpaikallaan. Puoliloiskasvit ottavat juuriyhteyksiensä avulla isännistään ravinteita sekä vettä ja siten rasittavat isäntiään itse tilanteesta hyötyen. Loisen ja isännän välillä vallitsee siis kilpailuasetelma. Kokeessani tutkin hypoteesia, ovatko kunkin ahosilmäruoho-esiintymän puoliloiset paikallisesti sopeutuneet hyödyntämään oman esiintymäpaikkansa isäntiä vai kykenevätkö ne yhtä hyvin hyödyntämään muualta peräisin olevia isäntäyksilöitä. Laboratorio-olosuhteissa suoritettua ruukkukasvatuskoetta varten keräsin siemeniä ja kasvatin puoliloisia sekä isäntiä kolme kuukautta samassa ruukussa. Puoliloinen sai seurakseen samaan ruukkuun isännän taimen joko suoraan puoliloisesiintymästä tai sen ulkopuolelta. Tulokset, jotka perustuivat puoliloisen ja isännän kasvun analysointiin osoittivat, että paikallista sopeutumista ei juurikaan ollut havaittavissa uhanalaisen ahosilmäruohon ja sen isäntäkasvin välisessä suhteessa. Tämä mahdollistaisi uhanalaisen puoliloisen istutukset uusille kasvupaikoille, koska puoliloisen isäntäkasvivaatimukset eivät ole rajoittuneita.

Väitöskirjani viidennessä osatutkimuksessa tutkin mäkitervakolla geneettisen monimuotoisuuden, populaatiokoon ja yksilön kelpoisuuden välisiä suhteita lajin äärilevinneisyysalueen isoiloituneissa (Jyväskylän seutu) ja päälevinneisyysalueen (Tampereen seutu) populaatioissa. Geneettisellä monimuotoisuudella tarkoitetaan yksilön perimässä olevan vaihtelun määrää, joka samalla muodostaa populaation geneettisen vaihtelun määrän. Muuntelun määrää mitattiin entsyymielektroforeesi-menettelmällä kasvin lehtinäytteistä. Yleensä oletetaan, että pienissä ja isoiloituneissa populaatioissa on vähemmän geneettistä muuntelua. Tulokset osoittivat, että isoiloituneiden populaatioiden välillä oli vain vähän geeninvaihtoa. Esiintymät olivatkin hyvin erilaistuneita toisiinsa

verrattaessa. Äärilevinneisyysalueen populaatioissa oli vähemmän muuntelua kuin päälevinneisyysalueen populaatioissa. Oletusten mukaan pienissä esiintymissä oli vähemmän muuntelua kuin suurissa esiintymissä. Tutkin myös idätys- ja kasvatuskokein vaikuttaako muuntelun määrä ja populaatiokoko kunkin esiintymän siementen keskimääräiseen itävyyteen, taimien kasvukykyyn ja kasvien siementuottoon. Populaatiokoko ja muuntelun määrä eivät kuitenkaan vaikuttaneet ko. ominaisuuksiin, vaan myös pienten ja vähän muuntelevien esiintymien yksilöt kykenivät tuottamaan hyvin itäviä siemeniä ja kasvukykyisiä taimia, hyvän siementuoton lisäksi. Nämä yksilöiden kelpoisuutta kuvaavat muuttujat eivät eronneet ääri- ja päälevinneisyysalueen populaatioiden välillä. Tulokset osoittivat, että suojelutoimia kannattaa kohdistaa myös levinneisyysalueensa äärirajoilla oleviin, isoituneisiin, esiintymiin. Pidemmällä aikavälillä juuri näissä ääripopulaatioissa saattaa syntyä uusia, olosuhteisimmee erityisen sopivia ominaisuusyhdistelmiä, jos vaan esiintymissä on muuntelua jäljellä luonnonvalinnan toimintaa varten.

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