Stockholm University

Studies on population biology and reproductive patterns in the genus *Pulsatilla* (Ranunculaceae)

Torbjörn Lindell

Doctoral Thesis in Ecology and Evolution at Stockholm University, Sweden 2026

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Abstract

An increasing number of plant species are threatened, usually as a result of human activities not targeted at the species as such. These activities could either affect the species immediately, such as radical land use change, or during an extended period of various length, often covering decades, such as gradual climate change. During these circumstances long-term preservation of an endangered plant will not be obtained only through protection of the species by law. Instead, conservation measures must focus on the environmental conditions affecting the functional unit, the population, and its conservation or restoration. To do so successfully several aspects of population biology can contribute with essential information for short term as well as long-term conservation measures. In this thesis I have selected to work with different aspects of the population biology of the genus *Pulsatilla*, which has a slow-paced life history with four out of five taxa in decline in the Nordic area. I selected to work with taxonomy, phenological patterns, demography, influence of climatic variables, breeding systems, crossing capacity within and between populations, optimal crossing distance and competitive ability. For this purpose I performed a series of field and experimental investigations of various lengths and, for the taxonomical part, I also collected data from herbarium specimens. The results show that the uniform comparison of morphological characteristics of specimens from the entire Nordic area has altogether clarified the status for each taxon regarding morphology and has also added taxonomical information within the *Pulsatilla vulgaris* group. Reproductive success was significantly, but not uniformly, influenced by temperature in *Pulsatilla vulgaris* subsp. *vulgaris* and in *P. pratensis*. Fecundity may depend on pollen limitation, parasites and resource allocation during part of the periods of flowering and growth as well as if the two species grow together or not. Climatic conditions during the previous summer and autumn affected flowering in such a way that high precipitation and low temperatures resulted in higher incidence of flowering but no significant effects on growth and survival from climatic variables were found. The variation in flowering had only modest absolute effects on the population growth rate but simulated persistent changes in average climatic variables gave substantial reductions in population sizes. Breeding systems varied within the genus but the potential for self-fertilization is high both in *Pulsatilla patens* and in the *Pulsatilla vulgaris* group. Hybridization barriers between taxa within the *Pulsatilla vulgaris* group are weak or non-existing. Resource limitation is most likely important in determining reproductive success. There were differences in fitness in the offspring depending on the distance between the parental plants. An optimal outcrossing distance of 1 km was found in *Pulsatilla vulgaris* subsp. *gotlandica*. No differences in competitive ability depending on crossing distance were found.

From a conservation perspective this thesis has added knowledge essential for risk analysis and planning for long term survival of herbs with a slow-paced life history exposed to climate change and habitat fragmentation. It has through long-term studies extending over several decades provided more solid predictions of the effects of climate change than would have been possible with shorter subsets of data. The thesis has contributed to increased knowledge of phenological patterns as well as influence of temperature and precipitation on vital rates, important for vulnerability analyses on a population level. In addition, analyses of breeding systems and the influence of crossing distance within a genus exposed to habitat fragmentation have added valuable insights for conservation purposes, since the results will give information about the capacity of a population to produce vigorous offspring.

Keywords: *Pulsatilla, Anemone, perennial herbs, flowering, phenology, plant demography, climate change, breeding systems, crossing capacity, population dynamics, integral projection model, vital rates, outcrossing distance, endemic, precipitation, temperature.*

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STUDIES ON POPULATION BIOLOGY AND REPRODUCTIVE PATTERNS IN THE GENUS *PULSATILLA* (RANUNCULACEAE)

Torbjörn Lindell

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Cover image: *Pulsatilla vulgaris* subsp. *gotlandica* photo by Torbjörn Lindell

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"Semper aliquid novi e Gotlandia"
Elias Fries
(On the discovery of *Pulsatilla patens* as a new species for Sweden)

The following five papers are included in the thesis:

I Lindell, T. 2001. *Pulsatilla* Mill. In B. Jonsell & T. Karlsson (Ed.), *Flora Nordica* 2 pp. 305-310. The Bergius Foundation. - The Royal Swedish Academy of Sciences.

II Widén, B. & Lindell, T. 1995. Flowering and fruiting phenology in two perennial herbs, *Anemone pulsatilla* and *A. pratensis* (Ranunculaceae). *Symbolae Botanicae Upsalienses* 31: 145-158.
- BW and TL contributed equally to study questions, field work, data analysis, visualization, writing and revisions.

III Lindell, T., Ehrlén, J. & Dahlgren, J. P. 2022. Weather-driven demography and population dynamics of an endemic perennial plant during a 34-year period. *Journal of Ecology* 110: 582-592.
- T.L. designed and conducted the field study; T.L., J.E. and J.P.D. conceived the study questions; T.L. and J.P.D. analysed the data; T.L. wrote the first draft of the manuscript and all authors contributed to revisions.

IV Lindell, T. 1998. Breeding systems and crossing experiments in *Anemone patens* and in the *Anemone pulsatilla* group (Ranunculaceae). *Nordic Journal of Botany* 18: 549-561.

V Lindell, T., Dahlgren, J. P., Ehrlén, J. & Widén, B. 2026. Outcrossing distance and reproductive success in the endemic perennial herb *Pulsatilla vulgaris* subsp. *gotlandica*.
- Manuscript. T.L. and B.W. designed the study; T.L., B.W. and J.E. conceived the hypotheses; T.L. conducted the field study; T.L., J.E. and J.P.D. analysed the data; T.L. wrote the first draft of the manuscript and J.E. and J.P.D. contributed with revisions.

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Introduction

Background

Anthropological influence on habitats in the form of land use change has been altering the conditions radically for many plant species over a long period and fragmentation of suitable habitats has developed into a severe threat to many species (e.g. Foley et al 2005, Song et al. 2018, Tsiftis et al. 2025). In recent years human-induced influence on climatic factors has been added as an important environmental ´driver´ influencing population dynamics and biodiversity (Sala et al. 2000, Montrás-Janer et al. 2024 and references therein). From this follows that a major task for ecologists is to assess different aspects of the population ecology of threatened taxa to better understand the environmental ´drivers´ behind their population dynamics and thus the most important threats, which is fundamental for efficient conservation measures (Oostermeijer et al. 2003, Ehrlén & Morris 2015 and references therein). These studies should include both short term as well as long-term aspects to cover also rare and extreme conditions which may influence vital rates during the life cycle. For conservation purposes the short-term studies are important since they establish rapid baseline demography, identify acute population declines or urgent threats and, if conservation measures have been tested, they provide information about the outcome and early life-cycle benchmarks of e.g. translocation or fencing. Long-term studies, on the other hand, are needed to document actual population persistence, adaptation capacity across generations and genetic resilience. Furthermore, they reveal multi-year ecological shifts, capture climate change impact including infrequent or extreme events and slow-moving habitat degradation (Compagnoni et al. 2021, Bialic-Murphy et al. 2022). In addition, a deeper understanding of how evolutionary processes interact with the ecological environment may also be important for the preservation and management of biodiversity under rapid environmental change (Hu et al. 2025). Finally, taxonomy provides the foundational framework for conservation since that will give the tools to identify, name and prioritize endangered species accurately.

Plant conservation aspects of land use change

Habitats in the northern hemisphere exposed to loss of area due to land use change include environments as diverse as short vegetation ecosystems and open forests (e.g. Sala et al. 2000, Luoto et al. 2003, Helm et al. 2006, Plieninger et al. 2016). A consequence of land use change is the fragmentation and subsequent geographical and genetical isolation of plant populations. Small isolated populations of outbreeding species have reduced gene pools compared with larger populations and inbreeding problems may occur together with loss of genetic diversity in general (e.g. Honnay & Jacquemin 2007, Aguilar et al. 2019). The consequences of such isolation may be extinction of a threatened species through destroyed metapopulation dynamics or genetic depletion (e.g. Aguilar et al. 2018 and references therein). It should also be noted that the influence of fragmentation on biodiversity in general has been under debate regarding fragmentation effects in relation to time and scale. (Helm et al. 2006, Krauss et al. 2010, Aguilar et al. 2018, Fletcher et al. 2023 and references therein). Especially challenging is to assess the consequences of fragmentation for perennial herbs with a slow-paced life history and thus a slow generation turnover (Tsuzuki et al. 2022 and references therein). Consequently, it is of utmost importance for conservation efforts for such species that the actual breeding systems and the fitness of the offspring produced from different crossing combinations and distances are known since that will provide information about a population´s genetical health, reproductive viability and risk for extinction. If, for instance, an optimal

crossing distance is found that will give a ´sweet spot ´for conservationists, a distance where the offspring vigor is maximized.

Plant conservation aspects of climate change

In recent years the question about how climate change affects the distribution and abundance of species has been accentuated due to increased anthropogenic influence. A key to obtain information about how climate change affects a threatened plant species is long-term demographical studies of vital rates which, in connection with parallel measurements of temperature and precipitation, is of the utmost importance in conservation since such studies will tell if a population is growing, stable or declining. Contrary to what is common in plant demographic models, which often focus on how vital rates are affected by stochastic factors, the demographic studies in relation to climatic factors must focus on a directional change since the variation of the climatic factors is not around a constant mean value (Ehrlén & Morris 2015). Several population studies have been performed with focus on conservation of rare perennial plants exposed to climate change (e.g. Lendvay & Kalapos 2014 and references therein). However, little is known about the long-term effects of climate change on population dynamics since this requires population studies extending over decades to capture also rare and extreme conditions in the variation of temperature and precipitation but most studies are considerably shorter (Morris et al. 2020).

In addition, climate change also affects phenological patterns within a species. These changes in phenology may in turn affect interactions with both the abiotic environment and other species. Not only will vital rates such as onset and duration of flowering and fruit set be affected during climate change, the synchronization with interacting species such as pollinators or other species in the same habitat may be disconnected. This will affect mutualistic relationships as well as food webs and, in the long term, may even evolutionary trajectories be altered (e.g. Morellato et al. 2016, Fogelström 2019, Ettinger et al. 2022 and references therein). In addition, due to the breakdown of phenological barriers entirely new species may interact with the threatened species causing additional disturbances to vital rates. In order to include phenology in the development of conservation measures of threatened species during climate change it is essential to focus on research within the fields described above since that may give information about the sensitivity of a rare species to rapid environmental changes and how to protect mutualistic relationships and breeding system patterns to find the proper timing of interventions.

Aim of thesis

This thesis aims to increase the knowledge about the population dynamics in long-lived herbs exposed to habitat fragmentation which, if applied, could further facilitate efficient conservation measures. For this purpose I have selected to work with different aspects of the population biology and reproductive patterns in the genus *Pulsatilla*, a slow-paced perennial herb exposed to fragmentation, through a series of field and experimental investigations of various lengths but also through morphological studies of herbarium specimens for the taxonomical part. Selected aspects of interest for conservation purposes were taxonomy, phenological patterns, influence of climatic variables, demography, breeding systems, crossing capacity within and between taxa, optimal crossing distance and competitive ability. The studies focus primarily on the genus *Pulsatilla* but the aim is also to provide results of interest for decisions about conservation measures for other slow-paced long-lived perennial herbs (Fig. 1).

The key questions I wanted to answer were the following:

1. What is the current status for the genus *Pulsatilla* regarding taxonomy, morphology, past and present distribution and biology? (Paper I)
2. Is there variation in flowering and fruiting phenology among sympatric species within the genus *Pulsatilla*? Connected to this question are the following problems also important to consider for conservation purposes during climate change: Is there a correlation between flowering and fruiting time? Does pollen or resource limitation affect the seed set? How important is seed predation for fecundity? (Paper II)
3. How does among-year variation in climatic conditions affect vital rates and population growth rate in the past, present and future, and how does the duration of the study influence our ability to assess the effects of climatic factors on population growth rates? (Paper III)
4. Which breeding system patterns and hybridization barriers exist and how will these affect fitness in the offspring during different conditions? (Paper IV)
5. Are there any effects of different crossing distances on the offspring and, if so, when are these effects expressed during the life cycle and, in addition, will competitive ability vary between crossing distances? (Paper V)

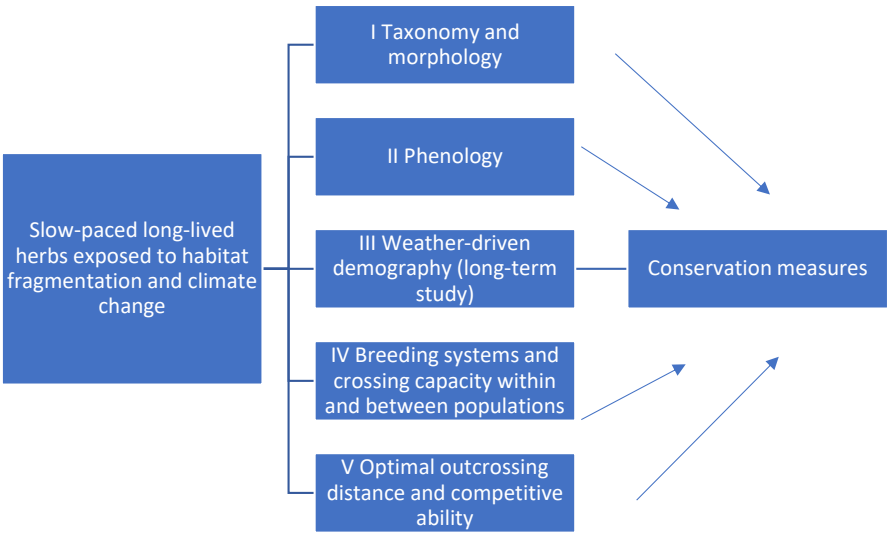

Figure 1. Overview of key concepts and papers included in this thesis.

Study system

The origin of this thesis goes back to 1983 as part of a project called 'Biological and genetical effects of habitat fragmentation (plants)' which was funded by the Swedish Environmental Protection Agency. Several genera with different life cycles were involved and I selected to work with the genus *Pulsatilla* (which then included in *Anemone*) which includes several taxa in decline in the Nordic area with populations of different sizes exposed to fragmentation. Already from the beginning both short term as well as long-term population effects were considered. The short-term studies focused on the

immediate survival of existing populations and the long-term studies had focus on the preservation of genetic diversity within the selected species and to secure evolutionary lines represented by the different taxa. Initially effects of climate change were not included but since the yearly demographical readings continued into a series covering decades, and at the same time the importance of the effects of climate change was more and more accentuated in the general scientific debate, climatic conditions became an important additional aspect. Over the years also the general scientific methodology altered when population research was used for conservation purposes. Initially field investigations in populations, e.g. demographical patterns within a species, was studied separately with 'vertical' applications often on a local level and not as part of an extensive and international network. However, with the development of statistics together with a rapidly developing ability to handle large amounts of data, the scale and approach to use population data for conservation purposes has altered dramatically during the study period and now it is possible to e.g. calculate population growth rate based on demographical data in a much more powerful way. Entire models, such as GLMMs and IPMs used in the two latest articles in this thesis, have been developed and may be used for conservation purposes (e.g. Hickinbotham et al. 2025 and references therein).

The perennial genus *Pulsatilla* has five taxa in Scandinavia: *Pulsatilla vernalis*, *P. pratensis*, *P. vulgaris* subsp. *vulgaris, P. vulgaris* subsp*. gotlandica* and *P. patens*. *P. vulgaris* subsp. *gotlandica* is endemic to Gotland where it occurs uncommonly in the SE part (Fig. 2). They are all diploids (2n=16) with the exception of *P. vulgaris* which is a tetraploid (2n=32) and may have an allopolyploid origin from *P. turczaninowii* and *P. alpina* (Sramko et al 2019). The genus consists of hemicryptophytes which have basal leaves forming a rosette. The flowers are terminal and solitary with petaloid sepals and short nectariferous petals. Stamens and carpels are numerous (Fig. 2) The plants grow in open to semi-open nutrient-poor areas on sand, sandy moraines or limestone pavement with thin soil but also in light-open pine forests (Fig. 3). No seed bank exists but the established individuals can be very old. A study of *P. vulgaris* have found that the life expectancy for ten year old plants was between 27 and 50 years (Edelfeldt et al. 2019). With the exception of *P. vulgaris* subsp. *gotlandica* all taxa have had their overall distribution area reduced during the 20th century (Fig. 2), mainly through land use change, and a number of conservation measures have been suggested for taxa within the genus (Lindell 2007, Gargiulo et al. 2019, Zabicka et al. 2022, Klavina et al. 2025).

Figure 2 (next page). Images with distribution maps of all Nordic taxa included in the genus *Pulsatilla.* ● = Native; •= Native, very rare; †=Extinct; ʘ=Introduced, rare; ▼ = Native, decreasing rapidly. Distribution maps from Lindell (1996).

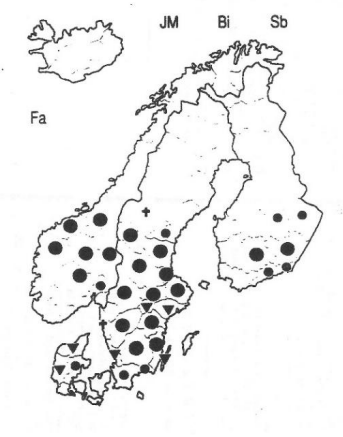

Figure 2A. *Pulsatilla* vernalis Photo: Torbjörn Lindell

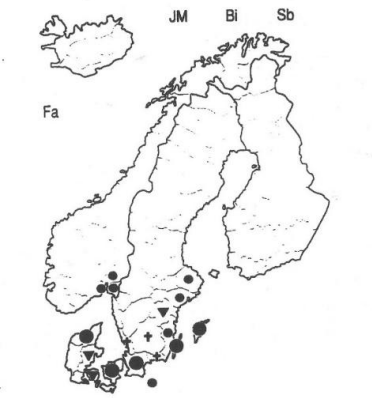

Figure. 2B. *Pulsatilla pratensis* Photo: Jörgen Petersson

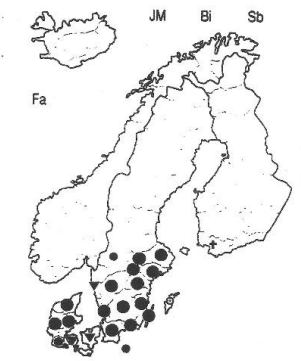

Figure. 2C. *Pulsatilla vulgaris* subsp. *vulgaris* Photo: Torbjörn Lindell

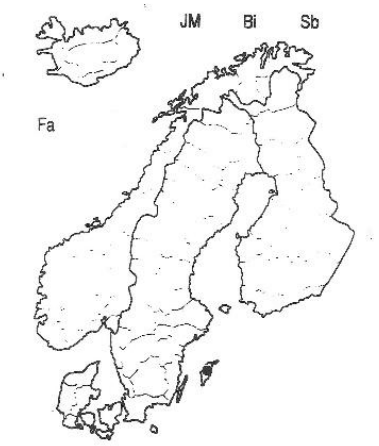

Figure. 2D. *Pulsatilla vulgaris* subsp. *gotlandica* Photo: Torbjörn Lindell

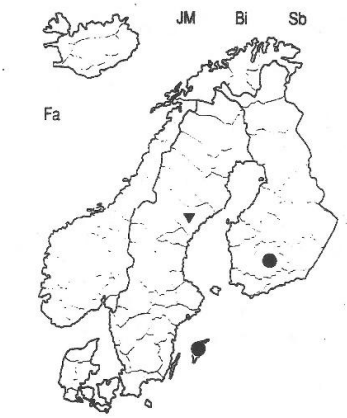

Figure. 2E. *Pulsatilla patens* Photo: Jörgen Petersson

Figure 3. Habitat of the endemic taxon *Pulsatilla vulgaris* subsp. *gotlandica* Photo: Torbjörn Lindell

Methods

A general description of morphology, distribution, history and biology of the genus *Pulsatilla* in the Nordic area (Paper I)

In order to understand the status regarding morphology, distribution, history and biology of Nordic taxa information was collected from herbarium specimens from the entire past and present distribution area of each taxon in the Nordic area in order to produce accurate distribution maps and uniform morphological information based on new measurements. Measurements on fresh specimens of *Pulsatilla patens* and *P. vulgaris* were also included. Floral as well as vegetative parts were measured and compiled in accordance with instructions given to all authors involved in the *Flora Nordica* project to guarantee uniform sets of data for all included taxa in the work. Upgraded information about present and past status for each taxon regarding distribution areas was also compiled from recently published papers, personal observations and contact with other botanists. Breeding system information was obtained from personal observations and experiments performed by the author. Habitat information was based on personal observations as well as literature references. The biological as well as the morphological data were finally used for taxonomical conclusions.

Patterns of flowering and fruiting phenology in *Pulsatilla vulgaris* and *P. pratensis* (Paper II)

To understand the pattern of flowering and fruiting phenology I studied two populations of *Pulsatilla vulgaris* subsp. *vulgaris* and one population of *P. pratensis* (sympatric distribution with one of the two *P. vulgaris*-populations) which were followed *in situ* and in cultivation during the years 1983, 1985, 1987-89. The development of individual flowers was followed from they were newly opened until the fruit set was completed. Daily average and maximum temperatures were recorded from March to June. The development of the individual achenes was recorded as well as any damage from predators. An additional experiment with hand-pollination on plants growing close to the plants in the phenology study was performed in order to investigate pollen-limited fecundity.

Influence of climatic variables on the population dynamics of *Pulsatilla vulgaris* subsp. *gotlandica* in a short and long-term perspective respectively (Paper III)

In order to understand the population dynamics in relation to climatic variables, demographic data were collected each year during a 34-year period in permanent plots in three populations together with yearly records of temperature and precipitation. Diameter, number of shoots, flowering and stem length were recorded from every individual present in the plots. New individuals were identified and followed each year. The species present in the bottom and field layers were also recorded. Vital rate models were fit using generalized linear mixed effect models (GLMM). In order to analyse the present trend in population growth and the effect of among year variation in precipitation and temperature on population growth, size-structured integral projection models (IPM:s), which included regression models of vital rates, were developed. The length of the study was also used to develop models to predict future population developments and also to analyse models with shorter simulated time spans to determine the length of time series needed to obtain enough data to accurately predict effects of precipitation and temperature on flower number and population growth rate.

Breeding system patterns and hybridization barriers in *Pulsatilla patens* and in the *Pulsatilla vulgaris* group (paper IV)

To understand breeding systems and to measure reproductive success from crossings within and between populations I used primarily Swedish material taken from geographically isolated natural populations which were collected as seeds during the period 1983-1985. Extra-Swedish material collected in natural populations was obtained from various botanical gardens. The seeds were sown separately within a few months after sampling since they rapidly lose their viability. After 2-4 months the plants were transferred to higher pots in order to facilitate the development of the tap root. The plants were arranged outdoors in a randomized block design in experimental beds after the first winter during which they were kept in a greenhouse. Usually, the plants did not flower until after four years. Bolting plants used in the crossing experiments were transferred to a greenhouse. Cultivation experiments including artificial pollination were used to map compatibility patterns and crossing barriers. Plants used in experiments were bagged and emasculated prior to pollen presentation.

Pollen viability was tested with cotton blue on two hundred pollen grains from each analysed flower. When possible F1-families were tested for fruit set, seed set and germination. In addition, a subsample of plants from each taxon and geographical group was used for morphological measurements.

Influence of crossing distance on the fitness of the offspring in *Pulsatilla vulgaris* subsp. *gotlandica* (Paper V)

To understand if an optimal crossing distance existed and if there were any inbreeding or outbreeding effects on the offspring in any part of the life cycle, I performed field crossings in seven different categories (passive selfing, active selfing, 1 m, 10 m, 100 m, 1 km and 3 km. All crossings for the six shorter distances were performed within the largest population, only the distance 3 km was performed between the largest and the second largest population. Eight different fitness components were analysed: fruit and seed set in the parental generation and germination, seedling development, development time, size, survival and flowering in the F1-generation after four growing seasons in cultivation in order to cover several phases of the life cycle. Two different sets of pots were used in the study, one set with siblings and one with achenes from all crossing categories to also allow analyses of differences in competitive ability between crossing categories (Ehlers & Bilde 2019 and references therein). These analyses were performed on plants of two and three years of age on the fitness components survival and size.

Figure 4. *Pulsatilla vulgaris* subsp. *gotlandica* and *Bombus pascuorum.* Photo: Torbjörn Lindell

Results and discussion

A general description of morphology, distribution, history and biology of the genus *Pulsatilla* in the Nordic area (Paper I)

I found that the morphological measurements supported the presence of two subspecies of *Pulsatilla vulgaris* in the Nordic area, subsp. *vulgaris* and subsp. *gotlandica*, because there were significant differences in several morphological characteristics when the two subspecies of *P. vulgaris* were compared. However, the breeding system studies, with no findings of sterility barriers, did not support a further division. During the work with the herbarium specimens, I designated the lectotype of *Pulsatilla vulgaris* subsp. *gotlandica*, however initially included in the genus *Anemone* (Jonsell 1996)

Furthermore, the compilation of the history and distribution of each taxon verified the overall decline in distribution. A deviating result from field observations was obtained regarding the Hymenoptera pollination of *Pulsatilla vulgaris* compared with observations on the continent. The dominating pollinators of *Pulsatilla vulgaris* subsp. *gotlandica* were species included in the genus *Bombus* (bumblebees) (Fig. 4), a result deviating from an extensive study on the continent by Kratochwil (1988).

Patterns of flowering and fruiting phenology in *Pulsatilla vulgaris* and *P. pratensis* (paper II)

I found a significant positive correlation between onset of flowering and date of fruit maturation. Early flowers needed a longer time to mature compared with late flowers. There was a difference in fecundity in the area where *Pulsatilla vulgaris* and *P. pratensis* were sympatric. At this site intermediate flowers of *Pulsatilla vulgaris* were more fecund than early or late flowers while late flowers of *Pulsatilla pratensis* tended to be most fecund. The low fecundity in early and intermediate flowers of *Pulsatilla pratensis* may depend on interspecific competition of pollinators since the larger and more conspicuous flowers of *Pulsatilla vulgaris* may be more attractive to pollinators than the smaller flowers of *Pulsatilla pratensis* (cf. Zimmermann 1980). In addition, I found that in cultivation the onset of flowering differed significantly among years between the two species with *P. vulgaris*-plants most exposed to sun were flowering first which indicates the importance of temperature for the onset of flowering. Overall, several observations within this study indicate that temperature is an important environmental driver regulating the rate of flower and fruit development both in *Pulsatilla vulgaris* and in *Pulsatilla pratensis*.

Regarding seed predation, I found that it was mainly by seed borers from two Diptera-families: Agromyzidae and Cecidomyiidae. The number of damaged ovules were 37% at most but no preference for either *P. vulgaris* or *P. pratensis* was observed. There was no seed predation in cultivation.

Isolation of individual flowers gave reduced fruit set in all populations but the pattern varied from no well-developed fruits at all in one of the populations of *Pulsatilla vulgaris* to a few well-developed in the other indicating differences in breeding systems within *P. vulgaris*. No well-developed seeds were found in isolated flowers of *Pulsatilla pratensis*.

Regarding pollen and resource limitation, I found no experimental evidence for pollen-limited fecundity in late flowers, but indications in early flowering plants. The significant correlation in the field between duration of fruit maturation and seed set indicates that more seeds mature in early flowers compared with late flowers since more time is available for the investment of resources in

seed development. In addition, germination rate was higher for seeds from early flowering plants compared with late flowering plants, which further indicates the importance of available resources. This availability may also depend on differences in allocation of resources within the individual plant at different times of the growing season since the leaf rosettes develop after the main flowering period. This may result in a competition between on one hand the allocation of resources to seed development and on the other the investment of resources in leaf development. Early flowers on the other hand, may suffer from lack of pollinators and may also be exposed to frost damage.

To summarize: There was a variation in flowering and fruiting phenology where *Pulsatilla vulgaris* and *P. pratensis* were sympatric. Focusing on the production of viable seeds, intermediate flowers of *Pulsatilla vulgaris* were more fecund than early or late flowers, but in *Pulsatilla pratensis* late flowers were the most fecund. I found no indication of pollen limited fecundity in late flowering plants but indications that this may occur in early flowers. The results indicate that resource limitation plays an important role for the seed set. Seed predation may affect fecundity to some degree in both species.

Influence of climatic variables on the population dynamics of *Pulsatilla vulgaris* subsp. *gotlandica* in a short and long-term perspective respectively (Paper III)

I found that flowering was clearly associated with climatic variables the previous summer and autumn, and increased after high precipitation and low temperatures. However, climatic variables did not significantly influence individual growth or survival. The weather-driven variation in flowering had only moderate effects on population growth rate during the 34-year period but simulation of future changes in average precipitation and temperature resulted in conspicuous changes in population sizes compared with the present status. Higher precipitation and lower temperatures were associated with higher population growth rates (Fig. 5). Simulations for the forthcoming 50 years resulted in positive effects on population sizes with increased precipitation while increased temperature had negative effects (Fig. 6). Still two out of three populations were predicted to decrease both in scenarios with climate change or without change in climate while the situation for the remaining population was more complex (Fig. 6). Analyses of subsets of flower number covering 5 or 10 randomly selected years resulted in estimated effects of temperature and precipitation which strongly deviated from the full data set (Fig. 7). Only when a subset of 20 year was used, the results were similar to the full data set. Overall, this study has emphasized the importance of collecting demographic data on a long-term basis in order to identify climatic drivers affecting vital rates and thus population dynamics in long-lived organisms.

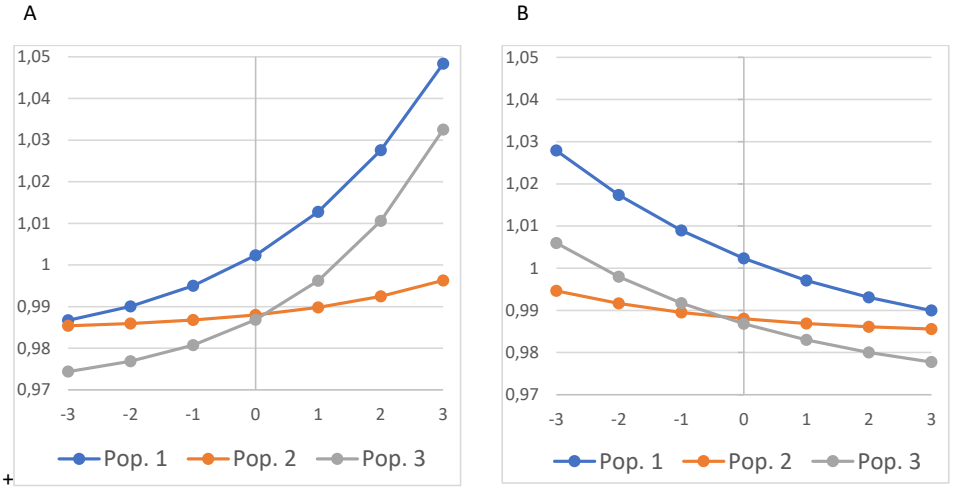

Figure 5. Deterministic population growth rate at major changes in precipitation (A) and temperature (B) up to 3 SD away from the mean during the study period. The y-axis shows population growth rate where 1 is stable population size; the x-axis shows standard deviations from the mean where 0 is the average conditions. Figure modeled on population data.

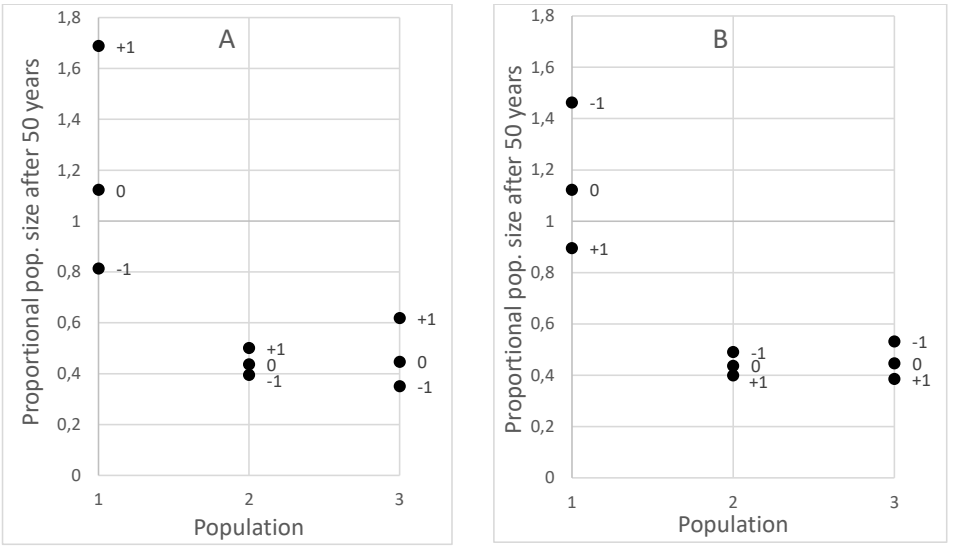

Figure 6. Population sizes after a simulated gradual change of precipitation (A) and temperature (B) up to one standard deviation away from the mean during a future 50-year period. The y-axis shows the proportional population size after 50 years where 1 represents the present size; the numbers on the x-axis represent the three investigated populations of *Pulsatilla vulgaris* subsp. *gotlandica* (dots = mean±1 SD). Figure modeled on population data.

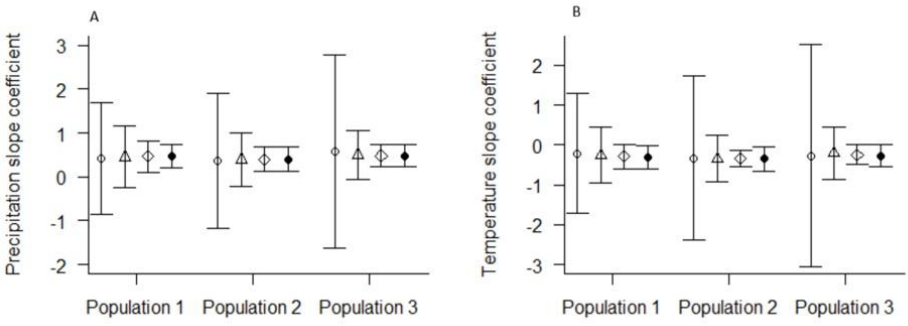

Figure 7. Influence of precipitation (A) and temperature (B) on yearly flower number. The y-axis shows the slope coefficients from regression analyses for expected change of flower number with changes in (A) precipitation and (B) temperature based on 10 000 randomly drawn subsets of data, using 5 (open circles), 10 (triangles) and 20 (diamonds) years, as well as coefficients estimated using 34 observed years (filled circles). Results are for three populations of *Pulsatilla vulgaris* subsp. *gotlandica*. For coefficients based on subsets, dots represent means and whiskers 95% of the range of simulated coefficients. For estimates based on all 34 years, whiskers represent the 95% confidence intervals.

Breeding system patterns and hybridization barriers in *Pulsatilla patens* and in the *Pulsatilla vulgaris* group (paper IV)

I found from the cultivation experiments that *Pulsatilla patens* and three subspecies of *Pulsatilla vulgaris* (ssp. *gotlandica*, ssp. *vulgaris* and ssp. *grandis*) were all self-compatible. Fruit and seed set were usually low after auto-deposition of pollen but increased after artificial self-pollination. The self-compatibility was contradictory to results found by Wells & Barling (1971) and Zimmermann (1935). Neither herkogamy nor dichogamy prevents autogamy completely which is consistent with results found by Jonsson et al (1991). The level of fruit and seed set varied considerably among outcrossing combinations from an increase compared with artificial self-pollination to very low especially in one population of *P. vulgaris* subsp. *vulgaris*. However, no crossing barriers were found between different subspecies of *Pulsatilla vulgaris*. I also found a variation in reproductive success between years, which indicate that resource limitation is most likely an important factor for reproductive success. The studied taxa are sensitive to cultivation conditions which influence reproductive success significantly. Finally, significant differences in morphological characters between the different subspecies of *Pulsatilla vulgaris* were found.

Influence of crossing distance on the fitness of the offspring in *Pulsatilla vulgaris* subsp. *gotlandica* (Paper V)

I found no significant differences in fruit or seed set between crossing categories in parental plants. In the F1-generation, selfed progeny did not perform worse than outcrossed, but the results indicate that biparental inbreeding may reduce fitness. Significant differences were found in five out of six fitness components. The variation was non-monotonous but fitness tended to be highest at a

crossing distance of 1 km. Analyses of integrated fitness, composed of both size and survival during the two final years, also resulted in a crossing distance of 1 km expressing highest fitness (Fig. 8). No differences in competitive ability between crossing distances were found when patches composed of either siblings exclusively or a mixture of different crossing categories were compared at two or three years of age.

Overall, there seems to be an optimal intermediate outcrossing distance at 1 km, which does not correspond with the average flight distances of the bumblebee pollinators which are considerably shorter. This investigation illustrates the importance of knowledge about breeding systems for conservation purposes and accentuates the need to elucidate if the behaviour of pollinators and actual crossing distances correspond to optimal outcrossing distances. A progressively used, but also debated, method in conservation to increase the size of threatened populations is reinforcement by reintroduction of new specimens (e.g. Zabicka et al. 2022, Stewart et al. 2025). During such reintroductions, it may be of importance to also consider the concept of optimal outcrossing distance to optimize the quality of the specimen used.

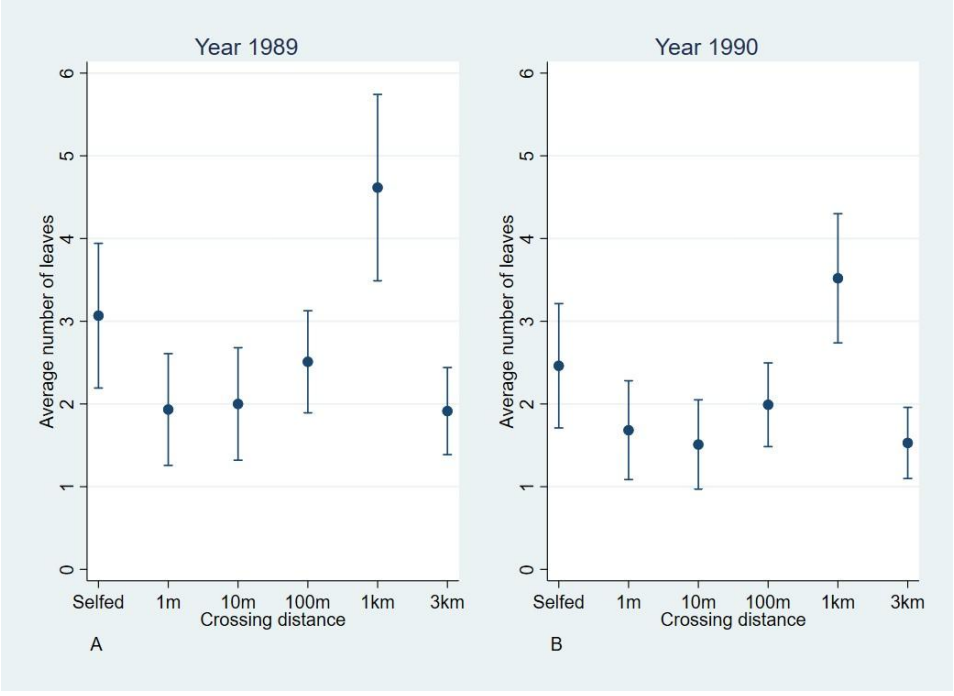

Figure 8. Integrated fitness in terms of size and survival in progeny from six different crossing distances grown together in pots with two seeds from each crossing distance during the third (A) and fourth (B) year. The y-axis shows the average number of leaves on individual plants where non-germinated seeds and dead plants were assigned a value of 0; the x-axis shows the distance between the parental plants in crosses. The dots show average values and bars 95 % confidence intervals.

Conclusions and future perspectives

The purpose of my studies was to increase the knowledge about the population dynamics in long-lived herbs exposed to habitat fragmentation which, if applied, could further facilitate efficient conservation measures. The thesis has also added knowledge essential for risk analysis and planning for long-term survival of herbs with a slow-paced life history exposed to climate change. Altogether the results may be a tool to carry out dynamic population viability analyses (PVAs).

An obvious conclusion from the results is that long-term studies and demographic monitoring are needed to cover also rare and extreme climatic conditions if solid predictions are going to be made about the future development of populations of long-lived organisms exposed to climate change. This is far from the present situation since demographic studies assessing the effects of climatic drivers only rarely extends over more than 10 years (Morris et al. 2020). This implies that our current knowledge about how plant population dynamics is affected by climatic factors is insufficient which may give unreliable predictions of the effects of future climate change. In addition, not only the number of years is important to correctly assess the influence of climate change on vital rates and population growth rates, the accuracy also depends on a sufficient number of individuals being followed. However, it is unlikely that larger sample sizes will substantially reduce the number of years needed to assess relationships. It will still be necessary with a long-term collection of data to capture the influence of rare and extreme conditions.

The thesis has also contributed to increased knowledge of phenological patterns. The phenological results have shown that temperature is an important environmental driver regulating the rate of flower and fruit development both in *Pulsatilla vulgaris* and in *Pulsatilla pratensis*. Climate change may alter the timing of these events and thus the synchronization with interacting species such as pollinators or other species in the same habitat which will disrupt mutualistic relationships and food webs. In addition, due to the breakdown of phenological barriers entirely new species may interact with the threatened species which may affect e.g. pollination, presence of parasites and herbivory. In order to include phenological research in the future in the development of conservation measures of threatened species during climate change, it is essential to focus on research within the fields described above. This may give information about the sensitivity of a rare species to rapid environmental changes and how to protect various essential interactions within and between species in the same habitat to find the proper timing of interventions. Such proper timing could include introduction of mowing or grazing based on phenological patterns to prevent resource depletion or structural damage. If climate change will disrupt the relationship between flower and pollinator, e.g. if flowering occurs before the pollinators emerge due to warming, then conservation measures could target microclimates, such as shaded patches or north-facing slopes, in order to delay flowering.

Analyses of breeding systems and influence of crossing distance on fitness of the offspring have added information important to consider for conservation purposes since such results will tell if it is likely that a population will be able to produce vigorous offspring. The studies of breeding systems, and the effects on the fitness of the offspring of various crossing combinations within and between populations, have revealed an optimal outcrossing distance which do not correspond with the actual flight distances of the most common pollinators. Habitat fragmentation may also interfere with the optimal outcrossing distance. For future conservation efforts it is therefore of importance to evaluate this discrepancy and consider if pollination should be facilitated in accordance with what was found to be the optimal outcrossing distance. Doing so will produce the most vigorous offspring to be used e.g. for reinforcement of existing populations or for the reintroduction of individuals in extinct populations.

Finally, in order to further contribute to future research I have, after 41 years of uninterrupted annual readings, handed over the fixed demography plots used in this thesis to a successor who will continue the series.

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Populärvetenskaplig sammanfattning

Ursprunget till denna avhandling går tillbaka till 1983 och projektet ”Biologiska och genetiska effekter av biotopfragmentering (växter)” vilket finansierades av Naturvårdsverket. Projektet startades eftersom att under det senaste århundradet den nordiska landskapsbilden i stor utsträckning har förändrats, något som också har skett på många andra håll i världen, vilket främst har berott på ändrade brukningsformer. Åtskilliga växter som tidigare fanns i större sammanhängande utbredningsområden har nu splittrats upp i flera mindre, något vi kallar biotop- eller habitatfragmentering. Detta kan innebära problem för vissa sällsyntare växter då populationerna inte bara blir geografiskt isolerade från varandra utan i många fall också genetiskt eftersom avståndet mellan populationerna har blivit för stort för att pollen skall kunna överföras mellan dem. På senare år har en annan viktig faktor kommit in som kan påverka sällsynta växters överlevnad och det är klimatförändringen. För att fatta rätt beslut om naturvårdsåtgärder för en viss sällsynt växt är det således viktigt att samla information om den ur olika aspekter som kan vara relevanta ur ett bevarandeperspektiv. Man utgår då från populationsstudier eftersom populationen, större eller mindre, är den fungerande enhet som arten uppträder i. Olika typer av populationsstudier är av intresse för att skapa en helhetsbild och ligga till grund för beslut som skall kunna hjälpa arten på sikt. Det är t.ex. inte säkert att samtliga befintliga populationer går att rädda, man kanske i stället måste överväga att försöka etablera arten på nya växtplatser. Viktig information om populationsbiologin kan man få genom att studera demografi, fenologiska mönster, fortplantningsmönster, effekter av korsningsavstånd och känslighet för klimatvariation. Olika växter har förstås olika krav på miljön för att kunna överleva.

Jag valde att i den här avhandlingen fokusera på populationsstudier inom släktet *Pulsatilla*, i vilket bland annat flera av våra sällsyntare sippor ingår, ett släkte som innehåller arter vilka har en livscykel som sträcker sig över lång tid och det är inte ovanligt att individer blir flera decennier gamla. Flertalet arter har minskat i antal inom det nordiska floraområdet till följd av fragmenteringen och åtgärder för att skydda hotade populationer är mycket angelägna. Det finns fyra arter inom släktet i Norden: mosippa, fältsippa, nipsippa och backsippa (*Pulsatilla vernalis, P. pratensis, P. patens* och *P. vulgaris*). Backsippa har dessutom två underarter inom utbredningsområdet nämligen subsp. *vulgaris* och subsp. *gotlandica*, den sistnämnda endemisk för Gotland och har fått namnet gotlandssippa. I avhandlingen har särskilt fokus lagts på studier av gotlandssippa vars skyddande således är uteslutande en svensk angelägenhet.

I den första artikeln har jag arbetat med en översikt över samtliga nordiska Pulsatilla-arter vad avser taxonomi, utbredningsmönster (både nutida och tidigare sådana), historik och biologi. Jag har använt mig av både pressat och färskt material och genomfört nya mätningar med avseende på morfologi och lagt till biologiska data bl.a. baserade på egna experiment. Själva upplägget på artikeln har styrts av att den ingår i ett floraverk och alla författare har fått detaljerade instruktioner att följa så att strukturen på respektive artikel blir uniform genom hela verket. Nya fakta om fortplantningsmönster och pollinationsbiologi har kunnat tillföras och ett tydliggörande av taxonomin inom gruppen har kunnat genomföras. Underartsindelningen av backsippa har fastställts och jag kunde också utse en lektotyp för gotlandssippa. En uppgraderad och detaljerad bild av utbredningen inom släktet kunde skapas och en tydlig bild av den delvis kraftiga minskningen i utbredning framträdde.

Den andra artikeln behandlar fenologiska mönster i blomning och fruktsättning hos två av arterna, fält- och backsippa. Dessa arter är delvis sympatriska d.v.s. de förekommer tillsammans. Två populationer av backsippa och en av fältsippa följdes både i fält och i odling. Det visade sig att de plantor av backsippa som var mest exponerade för solljus började blomma först och temperatur konstaterades vara en viktig faktor när det gällde påverkan på tidpunkten för blomningens inledning.

Tidiga blommor behövde längre tid för att sätta mogna frukter vilket således innebar att fruktmognaden gick snabbare längre fram under vegetationsperioden. Bäst fruktsättning hos backsippa hade plantor som blommade mitt under säsongen medan senblommande plantor av fältsippa hade bäst fruktsättning i områden där arterna möts. Sammantaget indikerar resultaten att resursbegränsning spelar en viktig roll för fruktsättningen. Möjligen spelar pollenbegränsning också en roll för fruktsättningen hos de plantor som blommar tidigt. Predation på frukterna förekom och var huvudsakligen orsakad av medlemmar av två familjer av tvåvingar (Diptera) nämligen Agromyzidae och Cecidomyiidae. Dessa åt inte frukterna rakt av utan larverna borrade sig in i frukten för att där äta av den vilket innebar att frukterna kunde verka helt oskadade men i själva verket var tomma.

I artikel tre användes en demografiserie i fasta provytor i tre populationer av gotlandssippa täckande hela 34 år för att analysera vilket inflytande variation i temperatur och nederbörd hade på ”vital rates” (= tillväxt, reproduktion och överlevnad) och populationsdynamik. Genom seriens längd kom även sällsynta tillfällen med extremvärden för temperatur resp. nederbörd med, något av stor betydelse med anledning av den pågående klimatförändringen. En tydlig påverkan på blomning orsakad av variation i klimatfaktorer kunde konstateras. Riklig blomning hade föregåtts av hög nederbörd och låg temperatur föregående sommar. Däremot kunde inte någon signifikant påverkan på individtillväxt och överlevnad konstateras vid variation i klimatfaktorer. Befintlig variation i klimatfaktorer hade endast en måttlig inverkan på populationstillväxt men simulerade varaktiga förändringar i medelvärdet för temperatur och nederbörd resulterade i betydande populationsminskningar vid en jämförelse med nutida förhållanden. Vidare så resulterade analyser med slumpmässigt utvalda klimatdata från perioder på fem resp. tio år i kraftiga avvikelser när det gällde att förutse påverkan på blomning jämfört med analyser utförda med data från hela den 34-åriga serien. Det var först när data täckande en 20-årsperiod användes som resultaten närmade sig det kompletta analysresultatet. Detta är intressant eftersom de allra flesta demogafiska serier håller sig inom ett intervall på 5-10 år. Det är således mycket viktigt att tillräckligt långa serier används för att identifiera de viktigaste klimatvariablerna när det gäller vad som driver populationsdynamiken hos långlivade växter under påverkan av klimatförändring.

Den fjärde artikeln fokuserade på fortplantningsmönster och korsningsförmåga hos nipsippa, backsippa och gotlandssippa liksom en utländsk underart av backsippa (*P. vulgaris* subsp. *grandis*). Det visade sig att samtliga taxa var självfertila men att frukt- och frösättningen i regel blev bättre vid korsbefruktning. Mönstret varierade dock mycket men några absoluta korsningsbarriärer kunde inte återfinnas vid korsningar mellan de olika populationerna och underarterna av backsippa. Troligtvis är resursbegränsning en avgörande faktor för reproduktiv framgång. Morfologin undersöktes hos de tre underarterna av backsippa och signifikanta skillnader mellan dem kunde konstateras för flera karaktärer.

I den femte och avslutande artikeln genomfördes ett försök för att fastställa ett eventuellt optimalt korsningsavstånd hos gotlandssippa d.v.s. ett avstånd mellan föräldraplantorna som ger avkomma med bäst livsduglighet. Bakgrunden till frågeställningen är att man hos vissa arter har hittat ett sådant avstånd men inte hos andra. Hos växter med dålig spridningsförmåga kan man anta att ju närmare individer växer varandra desto större är sannolikheten för att de är nära släkt. Då borde också inavelsrisken vara större vid korta avstånd mellan föräldraplantorna. Å andra sidan har det visat sig att om avstånden mellan föräldrarna är mycket stort kan den genetiska skillnaden vara så stor att avkommans livsduglighet minskar av den anledningen. Hos sådana arter har man kunnat påvisa att ett intermediärt korsningsavstånd ger bäst livsduglighet. Frågan om optimalt korsningsavstånd har stor betydelse för arter utsatta för habitatfragmentering eftersom den belyser den potentiella livsdugligheten hos avkomma som produceras under nya förutsättningar. I försöket pollinerades

plantor enligt sju avståndskategorier från självpollinering till ett korsningsavstånd på 3 km. Avkomman såddes i krukor med 14 frön dels med två frön från varje korsningsavstånd, dels i homogena syskonuppsättningar. Avkomman följdes i fyra år och kontrollerades med avseende på sex livsduglighetskomponenter: groning, överlevnad, individuell utvecklingshastighet, överlevnad, storlek och blomning. Avkomma från självbefruktning visade inte sämre livsduglighet än avkomma från korsbefruktning. Bland de sistnämnda var det korsningsavståndet 1 km som visade störst livsduglighet hos avkomman medan korspollinering mellan individer nära varandra visade sämre livsduglighet än avkomma efter självbefruktning. Ur naturvårdssynpunkt är det värdefullt att avkomma från självbefruktning inte visar försämrad livsduglighet medan det är mer problematiskt att det optimala avståndet 1 km inte alls överenstämmer med de mycket korta flygdistanserna som den vanligaste pollinatören, humlor, uppvisar.

Ur ett bevarandeperspektiv har resultaten gett kunskap som kan användas för naturvårdsplanering som inkluderar riskanalyser när det gäller bedömningen av livsdugligheten på lång sikt hos populationer bestående av perenna örter med en livscykel som sträcker sig över lång tid och som exponeras för både habitatfragmentering och klimatförändring. Genom att de demografiska studierna har sträckt sig över flera decennier har de gett ett underlag för mer solida förutsägelser om effekterna av klimatförändringar än vad som hade varit möjligt med studier över kortare tid. Avhandlingen har också bidragit med ökad kunskap om fenologisk variation och också visat hur temperatur och nederbörd påverkar demografiska mönster, kunskap som är avgörande för sårbarhetsanalyser på populationsnivå. Vidare så har analyserna av fortplantningssystem och betydelsen av korsningsavstånd för avkommans livsduglighet gett kunskap viktig att ta hänsyn till vid genomförandet av dynamiska sårbarhetsanalyser av populationer för naturvårdsändamål.