Reproduction and genetics in fragmented plant populations

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General introduction

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GENETICS IN FRAGMENTED POPULATIONS

The decline of many formerly common plant species is a major concern in nature conservation. Human demands and activities are responsible for the fragmentation and deterioration of the natural habitats of our wild plant populations (Lande 1998). Numerous species are presently forced to live in small and isolated natural fragments either with or without formal protection. These new rare species are especially vulnerable to extinction, because their natural habitat and population size declined in a faster rate than their evolutionary potential can get adapted to. In this respect, they differ from species that are naturally rare, such as many endemics, which are often well adapted to small population size and low levels of genetic variation (Huenneke 1991).

Fragmentation of a population is often followed by a reduction of genetic variation, because the remaining sample of individuals only partly represents the original population. When populations remain small, these sampling errors cumulate. This random change in allele frequencies that occurs between successive generations is defined as genetic drift. In large populations these changes are only minor but in small populations they may be substantial, resulting in loss of non-selective alleles. Therefore, small populations generally maintain less genetic variation than large ones (Young et al. 1996). The reduction of heterozygosity associated with population bottlenecks is often small, because population growth restores heterozygosity, but not allelic diversity (Nei et al. 1975). Once alleles are lost, they can only return by mutation or migration. However, pollen and seed dispersal is increasingly hampered in the actual human-influenced landscape, because pollinator movements have become restricted (Westerbergh & Saura 1994, Kwak et al. 1998) and the diversity of dispersal processes of plants that was associated with traditional farming, has diminished (Fischer et al. 1996, Poschlod & Bonn 1998). Consequently, small populations face the risk of local extinction, because low levels of genetic variation reduce the adaptability to a changing biotic and abiotic environment (Barrett & Kohn 1991) and increase the susceptibility to pests and diseases (Beardmore 1983, Frankham 1995).

Besides reducing genetic variation, fragmentation enhances mating among relatives as the number of individuals within a population decline and gene dispersal between populations becomes restricted. Selfing is the most extreme form of inbreeding because it decreases heterozygosity by half each generation. The amount of self-fertilisation is depending on the interaction between floral morphology, the degree of self-compatibility and ecological factors, such as pollinator availability and plant density (Schemske & Lande 1985). Although selfing in self-incompatible plant species is genetically prevented (Richards 1986), inbreeding may still occur because neighbouring individuals are more related, owing to the restricted

dispersal of pollen and seeds (Schaal 1980b, Waser & Price 1983).

A negative consequence of inbreeding is the reduction in fitness, also known as inbreeding depression (Charlesworth & Charlesworth 1987). The interaction between inbreeding depression and mating system depends largely on the genetic basis of inbreeding depression. This genetic mechanism in natural populations is largely unknown, but two hypotheses have been reviewed by Charlesworth & Charlesworth (1987). In the dominance model, loss of fitness is due to increased expression of recessive or partly recessive deleterious alleles in the homozygous state, whereas the overdominance model is supposing superiority of heterozygotes over homozygotes at individual loci. However, a growing number of studies seem to support the (partial) dominance model instead of the overdominance model (Dudash & Fenster 2000). According to this model, natural selection against inbred individuals may purge deleterious recessive alleles from the population, reducing inbreeding depression. Populations with a long history of inbreeding are therefore expected to have lower levels of inbreeding depression. A survey of empirical evidence on inbreeding depression in plants conducted by Husband and Schemske (1996), showed indeed that selfing species expressed lower inbreeding depression than outcrossing species. From this point of view, purging the genetic load from populations by selection may be useful to restore population fitness. However, purging of highly deleterious alleles can cause immediate extinction of small populations with low reproductive rates, because of the added reduction in fitness from inbreeding (Wang et al. 1999). Purging in small populations will only be successful if inbreeding depression is due to partial dominance (Fenster & Dudash 1994). Nevertheless, the long-term survival is still at risk, because fixation of mildly deleterious alleles due to drift lowers the overall population fitness.

A possibility to restore population fitness is to perform inter-population crosses or increase population size by sowing of planting. However, mixing gene pools from different origins may lead to outbreeding depression, i.e., a reduction in offspring performance relative to the parents when genetically diverged gene pools are mixed. Outbreeding depression may be explained by an ecological mechanism and a genetical or physiological mechanism. (Templeton 1986, Dudash & Fenster 2000). The ecological mechanism is associated with the adaptation to local environment. Crosses between differentiated populations will dissociate genes associated with local adaptation, resulting in an offspring that is adapted to neither parental environment. The other mechanism involves the breaking up of coadapted gene complexes, like for instance the epistatic interaction among loci that enhances plant fitness. The opposite of outbreeding depression is heterosis, which is an increase of fitness relative to the parents after hybridisation, because of a large increase in heterozygosity (Fenster & Dudash 1994). In experiments it has appeared that heterosis may outweigh or balance the negative effects of outbreeding depression (Dudash & Fenster 2000).

REPRODUCTION IN FRAGMENTED POPULATIONS

The sessile habit of plants complicates gene flow within and between populations. Although many species are adapted to abiotic pollen vectors, like wind or water, a large number of species is dependent of insects, birds or other animals for their pollination (Proctor et al. 1996). This mutualistic relationship between pollinators and plants is important for the genetic and demographic functioning of plant populations (Vogel & Westerkamp 1991, Olesen & Jain 1994). However, habitat fragmentation reduces pollinator diversity and

disrupts plant-pollinator interactions (Rathcke & Jules 1993, Westerbergh & Saura 1994, Steffan-Dewenter & Tscharntke 1999). In addition, gene flow patterns will change or disappear as distances between plant communities become larger than the flight distances of the pollinators (Kwak et al. 1998). Populations may become too small, sparse and isolated for pollinators to find them (the Allee-effect), lowering seed set drastically (Jennersten 1988, Lamont et al. 1993, Groom 1998). However, co-flowering species may increase flower visitation and seed set of rare species in sparse populations (i.e. the facilitation effect (Oostermeijer et al. 1998)), but with increasing density of co-flowering species, competition for pollinators will lead to pollen limitation (Rathcke 1983).

A special form of the Allee-effect in plant species is the presence of a self-incompatibility system. Selfing in self-incompatible plants is genetically prevented. However, incompatibility may also occur when two outcrossed individuals are of the same mating type, having the same incompatibility or S-alleles (De Nettancourt 1977). To maintain a high cross-compatibility in populations, large numbers of S-alleles are necessary (Byers & Meagher 1992). However, in small populations drift may have reduced the diversity of S-alleles (Young et al. 2000), which may result in a lower mate availability and reduced seed production or even dissolution of self-incompatibility (Reinartz & Les 1994). In extreme situations, reproduction may fail completely, because populations may exist only of a single self-incompatible mating type (DeMauro 1993, Wilcock & Jennings 1999). Population persistence in these species may have been prolonged by vegetative reproduction, but from an evolutionary perspective they were already extinct.

Besides low seed production, another consequence of pollen limitation might be a shift of the mating system from predominant outcrossing to selfing (Harder & Barrett 1996), resulting in higher rates of self-fertilisation and (initially) increased levels of inbreeding depression (Lande & Schemske 1985, Charlesworth & Charlesworth 1987). Pollen limitation may result from either inferior pollinator service due to increased geitonogamy and intrafloral pollen deposition, or by insufficient pollination associated with pollinator limitation or absence. If pollinator service is infrequent, delayed selfing may provide reproductive assurance if cross-pollination fails (Lloyd 1992). However, unfavourable pollination conditions are likely to increase the amount of selfing, because (a) self-pollen has less competition from cross-pollen and (b) the degree of temporal and spatial separation between anthers and stigma may decrease in flowers that develop under poor conditions or stay unopened for a long period. The amount of autonomous self-fertilisation is influenced by both the degree of self-compatibility and factors as floral morphology and environmental pollination conditions (Lloyd & Schoen 1992). A shift from mixed-mating to selfing is only be possible if levels of inbreeding depression are low (Lande & Schemske 1985).

STUDY OBJECTIVES

Population size and mating system have a large influence on demographic and genetic processes in small and fragmented populations. By studying the pollination biology, reproductive success, mating patters, levels of genetic variation and individual plant fitness in declining and rare plant species, valuable information will be obtained on population viability and its persistence in the future. The comparison of species with a different life strategy and mating system will allow a better understanding of the ways in which they suffer from habitat fragmentation and adaptation to the changing environment.

In two threatened plant species with a different life-history, viz., the short-lived *Gentianella germanica* (Willd.) Börner versus the long-lived *Arnica montana* L., the following questions will be addressed:

1. What is the mating system of these species ?
2. Do small populations suffer from pollen limitation and reproductive failure ?
3. Is the degree of selfing higher in small population than in large ones ?

Considering the rapid decline of *A. montana* in The Netherlands over the last decades, and in addition to the above questions, we need information on different aspects of its population viability in order to understand the importance of genetic variation and pollination. In particular, we focused on the following questions:

4. Do smaller populations have a lower genetic diversity than large populations ?
5. Is there any evidence for a breakdown of the self-incompatibility system in small populations ?
6. Is offspring performance in smaller populations lower than in large ones ?
7. Is individual plant performance associated with heterozygosity ?
8. Is outbreeding depression an important consideration in population restoration ?

In addition, this information is important for the development of management programs for rare and threatened plant species. In general, the ecology of many rare species is rather well known, but a poor understanding of their population biology appears to be a bottleneck for effective conservation. This becomes readily apparent from a management and monitoring plan that has been written for *A. montana* in 1992 (Bokeloh & van Zanten, 1993), which clearly suffers from a lack of insight in the species' population structure and breeding system.

THE STUDY SPECIES

In this thesis, two plant species with a different life-history are investigated. *Gentianella germanica* Willd (Gentianaceae) is a strict biennial and self-compatible. *Arnica montana* L. (Asteraceae) is a long-lived perennial plant, which reproduces sexually by seeds and asexually by means of short rhizomes. In contrast to *G. germanica*, *A. montana* has turned out to have a self-incompatibility system.

In The Netherlands, both species are threatened and placed on the Red Data List (van der Meijden et al. 2000). They both occur in isolated patches of natural habitat or nature reserves located in a highly fragmented landscape surrounded by a heavily exploited agricultural matrix. Both species have their main distribution in Central Europe and occurrence in The Netherlands is in the western margin of the distribution area (Meusel et al. 1978, Hulten & Fries 1986).

Gentianella germanica has always been restricted to a few populations, because the species occurs in calcareous grasslands, which are only found in a very small part of the country. In this respect, *G. germanica* has always been rare in The Netherlands. *Arnica montana*, on the contrary, is a characteristic species of nutrient-poor grass-, heath- and moorland, which covered extensive areas in The Netherlands until the beginning of the twentieth century. Draining of the moorland, planting of pines on the waste grounds as a

provision of work and land reclamation are important causes for the deterioration and fragmentation of our heath- and moorland landscape. Besides these environmental consequences, the collecting of *A.montana* for medicinal purposes has also contributed to the decline in The Netherlands (Bokeloh & van Zanten 1993).

THE OUTLINE OF THIS THESIS

Following this introduction, this thesis contains six chapters. The first four analyse factors affecting population viability of *A. montana*. The last two chapters deal with aspects of the reproductive biology of *G. germanica* and its respons to fragmentation and management.

In CHAPTER 2 (REPRODUCTIVE SUCCESS AND CLONAL GENETIC STRUCTURE OF THE RARE *ARNICA MONTANA* (COMPOSITAE) IN THE NETHERLANDS), the breeding system and the clonal genetic structure in a medium-sized population are analysed. The outcome provides important knowledge for the interpretation of mating patterns, genetic structure, and the determination of population size.

In CHAPTER 3 (REPRODUCTIVE SUCCESS, OFFSPRING FITNESS, AND GENETIC VARIATION IN SMALL AND LARGE POPULATIONS OF *ARNICA MONTANA*) an analysis is made of the genetic variation and genetic population structure of 26 populations of various size. In a subset of 14 populations of different size, the natural seed production is examined and various fitness parameters are measured in the greenhouse to determine offspring performance.

These two chapters have shown that both S-alleles and pollinators are important for the reproduction of *A. montana*. Subsequently, in CHAPTER 4 (FLOWER VISITATION, OUTCROSSING RATE, AND THE RELATIONSHIP BETWEEN OFFSPRING PERFORMANCE AND INDIVIDUAL HETEROZYGOSITY IN *ARNICA MONTANA*) I investigated the hypotheses that reproductive failure is rather a consequence of low mate availabity than of pollinators, because most Asteraceae have a generalist pollination system. In contrast, we expect small populations to have more self-fertile genotypes than large ones, due to pollinator limitation. To test these hypotheses, we investigated the number of flower visitors and their visitation rates in relation to flowerhead density in one small and one large population. Pollen from the insect bodies was collected to estimate their role as pollinators. To determine the ability for selfing, we analysed seed set after artificial selfing in 11 populations of various size kept in the greenhouse. In three natural populations, two large and one small, outcrossing rates as well as the relationship between individual heterozygosity and plant size were analysed.

In CHAPTER 5 (DEMOGRAPHIC CONSEQUENCES OF INBREEDING AND OUTBREEDING IN *ARNICA MONTANA*: A FIELD EXPERIMENT), we compare the performance of selfed, intra- and inter-population crosses of plants introduced as seeds and as seedlings in a field experiment. Recently, much attention has been given to the restoration of the ecological habitat conditions to counteract the decline of several threatened plant species. However, demographic and genetic factors are other important factors determining the extinction of wild populations. Reintroduction of populations and restoration of genetically depauperate populations with individuals or seeds from other populations may be an important management tool to restore population viability in the very near future, but is largely unexplored for declining plant species.

Studies of the reproductive biological response to fragmentation and (changing) management of the short-lived, self-compatible *G. germanica*, are presented in chapters 6 and 7.

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In CHAPTER 6 (REPRODUCTIVE BIOLOGY OF THE RARE BIENNIAL *GENTIANELLA GERMANICA* COMPARED WITH OTHER GENTIANS DIFFERING IN LIFE HISTORY), we investigated the mating system in two large but highly isolated populations. In addition, a comparison of autofertility and ovule production per flower was made with several other gentian species differing in life history.

In CHAPTER 7 (VARIABLE HERKOGAMY AND AUTOFERTILITY IN MARGINAL POPULATIONS OF *GENTIANELLA GERMANICA* IN THE NETHERLANDS), we investigated changing patterns in the spatial and temporal separation of anthers and stigma in relation to autofertility. Data from 1998 are compared with data from 1991 and 1992. In between these years , one of the large study population was mown one month earlier than usual. This led to a dramatic reduction in population size (i.e., a bottleneck) and also strongly decreased pollinator abundance. The consequence of this man-induced population bottleneck on herkogamy (i.e. the spatial separation of anthers and stigma within flowers) is discussed in relation to selection for autofertility.