Reproduction and genetics in fragmented plant populations

Luijten, S.H.

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Summary

Many plant species have become rare and threatened due to loss and deterioration of their natural habitats, largely as a result of increasing human activities. Plant populations declined in size and often have to persist in small and isolated nature reserves. As a result, they are sensitive to local extinction, because of reduced genetic variation and offspring fitness due to genetic drift and inbreeding. In addition, small populations are vulnerable to reproductive failure as a result of reduced pollinator service and/or low mate availability. Besides population size, the species' mating system does have a large influence on genetic and demographic processes in small and fragmented populations.

This thesis reports on genetic and demographic aspects of two threatened plant species differing in life-history, viz. the short-lived *Gentianella germanica* and the long-lived *Arnica montana*. Both species are threatened and occur in isolated patches of semi-natural habitat in a highly fragmented landscape. However, *G. germanica* has always been restricted to a few populations, because it only occurs in calcareous grasslands, which are only found in the southernmost part of The Netherlands. In contrast, *A. montana* is a characteristic species of heathland, which covered extensive areas of The Netherlands until the beginning of 1900. Both draining and exploitation of heathlands and collecting of *A. montana* for medicinal purposes have contributed to the decline of this plant in The Netherlands.

In this thesis, a total of six studies is presented. The first four chapters analyse factors affecting population viability of the rapidly declining perennial *A. montana*. The last two focus on aspects of the reproductive biology of the biennial *G. germanica* and its response to management.

Knowledge on the spatial population structure and mating system are important necessities for accurately estimating population size and understanding demographic and genetical processes in declining plant populations. In a medium-sized population of *A. montana*, genotype analysis of all mapped rosettes in a plot of 100 m^2 (CHAPTER 2) suggested a clonal structure, because dense clusters often consisted of identical genotypes. However, open clusters frequently contained several different genotypes, due to limited fruit dispersal, since seedlings were found mainly within or in the near surroundings of the clusters. Pollination experiments showed that *A. montana* is largely self-incompatible. However, considerable variation in seed set after artificial self-pollination was observed in a small number of individuals, suggesting a partial breakdown or failure of the self-incompatibility system. Although some plants appeared to be more self-fertile than most others, pollinators are generally essential for pollination. Moreover, a high S-allele diversity is necessary to preserve cross-compatibility relationships.

Allozyme electrophoresis in 26 populations (CHAPTER 3) showed that total genetic variation was rather low in *A. montana* (H_e = 0.088). In addition, there were significant positive relationships between the log of population size and the proportion of polymorphic loci, the effective number of alleles and the level of gene diversity, which were attributed to genetic drift during bottlenecks. Observed heterozygosity was not related to population size. In general, small populations showed a heterozygote excess. It is possibe, that these individuals contained a relatively high fitness and represent the remaining survivors from the formerly larger populations. The *F*-statistics showed a moderately high level of differentiation among populations, implying a low level of gene flow.

Population size was also positively related with seed set (CHAPTER 3). It remained unclear if reproductive failure was due to pollinator limitation or to insufficient numbers of cross-

comptible mates. It is likely that drift has also eliminated S-alleles. Also several parameters of offspring performance (seedling size, the number of flowering stems and flowerheads, adult survival, and total relative fitness) were significantly reduced in smaller populations. However, offspring performance in the greenhouse was not associated with genetic diversity measured on their mothers in the field. We concluded that fitness of small populations is significantly reduced, but that there was no evidence that this was caused by inbreeding. Possibly, the self-incompatibility system of *A. montana* has been effective in reducing selfing rates and inbreeding depression.

The two preceding studies showed that fragmentation and small population size can strongly effect the reproductive success in species depending on pollinators or on cross-pollination. However, since most Asteraceae have a generalist pollination syndrome and are self-incompatible, it might be that members of this family may suffer less from pollinator limitation but more from mate availability. In contrast, pollinator limitation may increase the number of self-fertile plants due to selection for reproductive assurance, but higher levels of homozygosity associated with selfing might lower plant fitness.

An analysis of the flower visitors and their visitation rates in relation to flowerhead density in one small and one large population (CHAPTER 4) showed that, as expected, *A. montana* was visited by different insect groups, viz. hoverflies, 'other flies', butterflies, bees, beetles and bugs. All visitors carried heterospecific pollen, but considering the abundance, conspecific pollen loads and visitation rates of hoverflies and bees, we assumed that these visitors were the most efficient pollinators. Although the mean number of visits to a genet and the number of flowerheads visited per genet were similar between populations and plots, visitation rate per individual flowerhead was twice as high in a low density as compared to a high density patch of flowering plants. The small population received on average more visits per flowerhead per unit time.

No evidence was found for a higher proportion of self-fertile plants or a breakdown of the self-incompatibility system in small populations (CHAPTER 4). Moreover, high outcrossing rates in one small and two large populations suggested that self-incompatibility still functions properly. Interestingly was the higher number of pollen donors per flowerhead in the small population, which was in agreement with the higher visitation rate in that population, and additionally suggests that mate availability was still sufficient. Offspring performance was positively related with individual heterozygosity (CHAPTER 4), indicating that outcrossing is very important to maintain fitness.

Considering the rapid decline of *A. montana* during the last decades, genetical reinforcement of small populations from other populations by sowing or planting may be an important management tool to prevent this species from extinction in The Netherlands. However, a negative consequence of mixing gene pools might be the reduction of plant fitness as coadapted gene complexes are disrupted. In a four-year field experiment, demographic consequences of inbreeding and outbreeding in five populations of varying size were analysed on plants introduced as seeds and as seedlings (CHAPTER 5). Low cross-compatibility in one small population was responsible for a significant "heterosis" effect on seed set. Significant, but low, inbreeding depression was observed for growth rates of plants introduced as seedlings. We found significant heterosis for flowering probability of plants introduced as seeds, but for plants introduced as seedlings, heterosis for seedling size and flowering probability was only marginally significant. Although no outbreeding depression

was observed in the F_1, it may still be expressed in the forthcoming generations as recombination of genes start to break up the coadapted gene complexes associated with local fitness. It is not clear to what degree the observed heterosis may compensate this possible outbreeding depression. Nevertheless, considering the rapid decline of *A. montana* in The Netherlands, genetical reinforcement of the numerous small populations may be more important than the possible negative consequences of outbreeding depression. Another important argument to reinforce small populations genetically is the increase of S-allele diversity, which is important for the production of seeds.

Studies of changes in reproductive biology in response to isolation and management of the short-lived self-compatible *G. germanica* are presented in the last two chapters.

Pollination experiments in two populations of *G. germanica* (CHAPTER 6) show that this species is self-fertile, but that pollinators are essential for the transport of pollen to the stigmas. Although the mean seed set was relatively low (30%) in caged and unpollinated flowers, it varied considerably between individuals (0-90%), suggesting differences in the ability of plants to autodeposit pollen on their stigma. There was no evidence for pollination limitation in either population. The reduced seed set after hand selfing in one population indicates some inbreeding effects on ovule or seed abortion, but in the other population such inbreeding depression was not observed. A comparison of autofertility and ovule production per flower with several other gentian species differing in life history (CHAPTER 6) confirmed the hypothesis that the annual and biennial gentians are predominant selfers and that the perennials are predominant outcrossers. In contrast to other biennials, however, *G. germanica* was more similar to the perennial species, because of its poor autofertility.

In 1997 and 1998, we investigated the spatial and temporal separation of anthers and stigma in relation to autofertility (CHAPTER 7). Dichogamy appeared to be very weak and variable within individuals and is not expected to be an effective barrier against self-fertilization in this species. Herkogamy varied significantly between individuals and between populations, and was not correlated with plant and flower size. Autofertility was generally high in all three populations. Only in one small population a negative correlation between autofertility and herkogamy was found. Here, most plants had the stigma positioned above the anthers, while in the other two populations the stigma was positioned mainly in between or below the anthers. Comparing data from 1991 and 1992 with data from 1998, plants in one large population had become less herkogamous and showed higher autofertilitty. We suggested that the dramatic reductions (bottlenecks) in population size and pollinator abundance which were caused by changes in management have caused a selection for plants with a smaller anther-stigma separation and consequenctly a higher autofertility. Through this mechanism, human influence can have profound effects on the reproductive success and evolution of rare plant species, even in the context of nature conservation.

This thesis clearly shows that populations of formerly common, but currently rare plant species suffer from the fragmentation and deterioration of their habitat. Although there are some indications that populations of short-living species,like *G. germanica*, can adapt to the changing habitat conditions, evolutionary processes in long-lived species, like *A. montana*, may in the long run not be able to match the pace of habitat change, because of the loss of genetic variation and the reduced fitness associated with small population sizes. In such small populations, the the risk of extinction is increased and the ability to regenerate after ecological habitat restoration reduced. Hence, we should no longer wait, and increase our efforts to restore genetic variation and fitness of small populations, before it is too late !