

POLLINATION IN RARE PLANTS: IS POPULATION SIZE IMPORTANT?

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ABSTRACT: Habitat loss and fragmentation disturb and disrupt the interactions between plants and pollinators. The size of the remaining populations may be important in this context, as it can cause the Allee-effect and change pollinator behaviour. In this paper, we review our empirical data on the relationship between population size and reproductive success in some rare plants in The Netherlands, and compare our findings with the recent literature. The plants we studied differ in life history, breeding system and pollinator specificity. Most depended on insects for their reproduction, although the short-lived species were highly autofertile. In nearly all species, the relationship between fecundity and population size was significantly positive (except in the deceptive *Orchis morio*, where fruit set peaked at a population size of 40 flowering plants). However, other factors, such as plant density, facilitation by and competition with co-occurring heterospecifics, and light intensity were clearly important as well. A literature survey yielded a similar conclusion: population size and density are generally important for the reproductive success of animal-pollinated plants. Usually, this is caused by pollen limitation in small populations. In addition, genetic drift may have reduced the number of S-alleles in small populations of self-incompatible species, limiting the availability of compatible mates. In highly autofertile species, population size may be more related to the outcrossing rate than to seed production. Demographic consequences of reduction in seed production have been investigated only rarely. Yet, this is vital to assess the role of pollen limitation in the population viability of rare plants. The few studies that have been done suggest that in addition to reduced seed production, the inbreeding depression associated with the increased selfing rates is important for population viability in outcrossing species.

Det Norske Videnskaps-Akademi. I. Matematisk Naturvidenskapelige Klasse, Skrifter, Ny Serie, 39: 201-213 (2000)

INTRODUCTION

Due to large-scale habitat loss and fragmentation, many plant species formerly common in our landscape have now been reduced to scattered small and isolated populations. In

contrast to naturally rare species, such as local endemics, these formerly common plants can be seen as the "new rares" (Huenneke, 1991). At least during the last few hundred years, such species will generally have experienced larger population sizes and levels of

gene exchange than at present. As a result of genetic drift and inbreeding, small populations generally show a reduced genetic variability (Prober and Brown, 1994; Raijmann et al., 1994; Fischer and Matthies, 1998; Menges and Dolan, 1998; Ayres and Ryan, 1999; Young et al., 1999), which could in some cases be linked to a reduced demographic performance (Oostermeijer et al., 1994; Oostermeijer et al., 1995; Helenuurm and Schaal, 1996; Fischer and Matthies, 1997; Kephart et al., 1999). Some recent studies demonstrate that reductions in population size and gene flow will have negative impacts on the remaining populations, lowering population viability and increasing extinction risks (Newman and Pilson, 1997; Groom, 1998; Menges and Dolan, 1998; Oostermeijer, in press).

The interactions between plants and their pollinators are also important for population viability, as they do not only determine the quantity of the offspring, but also its quality, through pollen limitation and the degree of self-fertilization (Fægri and Van der Pijl, 1966; Olesen and Jain, 1994; Waser et al., 1996). Populations of rare plants may be too small to attract pollinators or to serve as a reliable pollen or nectar source (i.e. the Allee-effect, (Lamont et al., 1993; Groom, 1998)), which can lead to reduced visitation rates and an increase of (delayed) autogamous selfing. In addition, the generally low plant densities in small populations may affect pollinator behaviour, such as the amount of time spent on each plant. This may increase the rate of geitonogamous selfing (De Jong et al., 1993; Karron et al., 1995). However, the effects of reduced population size probably strongly depend on the pollination ecology and breeding system of the species in question (Olesen and Jain, 1994). Self-compatibility, prolonged floral longevity and shallow flowers are more frequent in rare than in common annual Brassicaceae in Israel (Kunin and Shmida, 1997), which supports the hypothesis that pollinator service is scarcer for rare

plant species.

In this paper, we present an overview of our empirical results on the relationships between population size and reproductive success for a number of "newly rare" plant species with different life histories and breeding systems. Comparing our results with the pertaining recent literature, we will try to answer the main question: is population size important for the pollination of rare plant species? To be able to interpret the results from studies into this relationship, we also need to address the results of pollination experiments to find out to what degree the species are dependent on pollinators for seed set and what effect selfing (or inbreeding) has on seed development.

SOME STUDIES ON RARE PLANTS IN THE NETHERLANDS

SPECIES STUDIED

Since 1987, when our project on the conservation biology of rare plants in The Netherlands started, we have gathered data on the demography, genetics and pollination biology of several species. An overview of important life history characteristics of the species on which we report here is given in Table 1. All of the species can be considered as "new rares", which were formerly common in the Dutch semi-natural landscape (Mennema et al., 1985) and are now threatened by habitat fragmentation. Most populations are small and found in isolated nature reserves. In some larger reserves where the traditional land-use has been maintained or restored by nature conservation organisations, large populations of these species can still be studied.

POLLINATION EXPERIMENTS

Bagging experiments to test the potential for autogamous selfing (autofertility *sensu* (Lloyd and Schoen, 1992) showed that the

Table 1. Some characteristics of the rare plant species studied in The Netherlands on which results are presented in this paper.

Species	Life history*	Main pollinators	Mean		Source
			fl/pl	ov/fl	
<i>Gentiana pneumonanthe</i> L.	LLP, IT	bumblebees	3	450	(Oostermeijer, 1996)
<i>Gentianella uliginosa</i> (Willd.) Börner	AN, SEM	bumblebees	10	60	(Petanidou et al., 1998)
<i>Gentianella germanica</i> (Willd.) Börner	BI, SEM	bumblebees	15	90	(Luijten et al., 1998)
<i>Arnica montana</i> L.	LLP, CL, IT	syrphid flies	720	1	(Luijten et al., 1996)
<i>Orchis morio</i> L.	LLP, IT	bumblebees	9	8400	(Kos et al. in review)
<i>Orchis militaris</i> L.	LLP, IT	solitary bees	23	n.d.	(Kos et al. in review)
<i>Orchis purpurea</i> L.	LLP, IT	bumblebees ?	47	n.d.	(Kos et al. in review)

* LLP = long-lived perennial, AN = annual, BI = biennial, CL = clonal, IT = iteroparous, SEM = semelparous; fl/pl = flowers per plant, ov/fl = ovules per flower, n.d. = not determined.

gentian species were able to set seed without pollinator visitation, although to a strongly variable degree (Figure 1a). In *O. morio* (Kos et al. in review) and *A. montana* (Luijten et al., 1996), exclusion of insects led to an extremely low mean seed set (Figure 1a). The highest autofertility was found in the annual *G. uliginosa* (Petanidou et al., 1998) and in the biennial *G. germanica* (Luijten et al., 1999), and autofertility was relatively low in the perennial *G. pneumonanthe* (Petanidou et al., 1995).

Manual self-pollination treatments on caged plants showed that all studied species were fully self-compatible, except for *A. montana*. Hence, in all cases where seed set in the autofertility test was lower than the seed set after the manual self-pollination treat-

ment, pollen limitation will occur in the absence of pollen vectors.

In most species, the comparison between manual self- and cross-pollinations revealed no significant pre- or postzygotic inbreeding. A significant reduction in seed set after self-pollination was only found in *G. germanica*, and, as expected, in the self-incompatible *A. montana*. In *G. uliginosa*, seed set after outcrossing was slightly lower than after selfing. In *A. montana*, the relatively low seed set after outcrossing can be attributed either to the fact that pollinations within a flower head (in which a ring of new florets opens every day) were carried out only every other day, which may have caused some pollen limitation, or to the cross-incompatibility of some acceptor-donor combinations (Luijten et al., 1996).

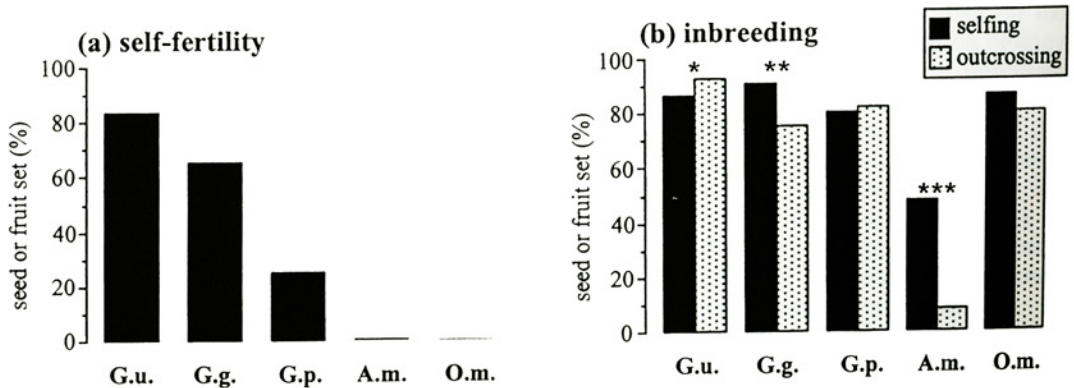


Figure 1. (a) Seed set after spontaneous self-pollination (autofertility) and (b) the difference in seed set after manual cross- and self-pollination (inbreeding) in five of the studied rare plant species. *G.u.* = *Gentianella uliginosa*, *G.g.* = *Gentianella germanica*, *G.p.* = *Gentiana pneumonanthe*, *A.m.* = *Arnica montana*, *O.m.* = *Orchis morio*. Asterisks in (b) indicate a significant difference between selfing and outcrossing in a species: * $P < 0.05$, ** $P < 0.025$, *** $P < 0.01$.

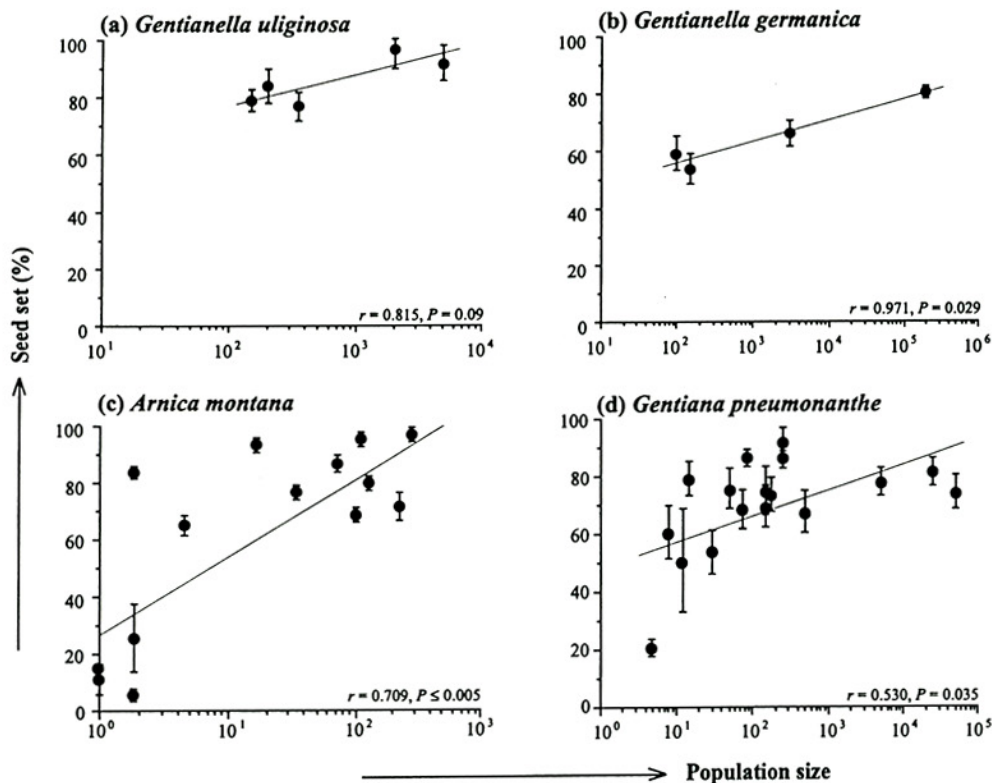


Figure 2. Relationships between population size (number of flowering plants in the population in the year of sampling) and seed set after natural pollination in four rare plant species in The Netherlands: (a) the annual *G. uliginosa*, (b) the biennial *G. germanica*, (c) the self-incompatible perennial *A. montana*, and (d) the self-compatible perennial *G. pneumonanthe*. Vertical bars represent the standard error of the mean; r in each graph is Pearson's correlation coefficient, accompanied by its significance value (P).

RELATIONSHIPS WITH POPULATION SIZE

Figure 2 shows the published relationships between population size and seed or fruit set in four of the species studied (data on the orchids will be published elsewhere). A significant positive relationship was observed only for *G. pneumonanthe* (Oostermeijer et al., 1998), *A. montana* (Luijten et al., in press) and *G. germanica* (Luijten et al., 1998;

1999). A marginally significant relationship was found for the short-lived species *G. uliginosa* (Petanidou et al., 1998). In the short-lived species, the differences in seed set between small and large populations were relatively small (10-20%, Figure 2).

No correlation between population size and fruit set existed in *O. militaris* (Kos et al., in review). In *O. morio* and *O. purpurea*, the relationship between population size and fruit set clearly differed from the other spe-

cies. In *O. morio*, there seemed to be an optimum fruit set of c. 70% at an intermediate (but still rather small) population size of 40 flowering individuals (Kos et al., in review). In populations of fewer than 40 plants, fruit set was less than 10% and increased with population size, decreasing again to 20% in the very large populations (>100,000 plants) of this species. In *O. purpurea*, there was a highly significant relationship between population size and fruit set when one of the studied populations was omitted. In this small population, the surrounding dense woodland had just been coppiced the year before sampling, which apparently increased insect visitation (Kos et al., in review).

COMPARISONS WITH OTHER STUDIES

THE IMPORTANCE OF POPULATION SIZE

Population size was an important factor for the reproductive success of most of the species studied. Species with high autofertility, such as *G. uliginosa* and *G. germanica*, are obviously less dependent on pollinators for their seed production than species which are either self-incompatible (*A. montana*), or fully self-compatible but poorly autofertile (*G. pneumonanthe*). The latter two species showed the clearest relationship with population size. In the two short-lived species, seed set in small populations was 10-20% lower than in the large populations. The fact that there is a significant increase with population size shows, however, that even in these highly autofertile species, pollinators may contribute significantly to seed production. In *G. pneumonanthe*, Oostermeijer et al. (1998) showed that other factors besides population size had a significant effect on seed set. The most important of these factors was the cover of co-flowering species such as *Calluna vulgaris* and *Erica tetralix*. These more common species can apparently attract pollinators (bumblebees in this case) to sites

with few gentians, thus increasing visitation rates. This facilitation effect (Rathcke, 1983; Kwak et al., 1998) has not been studied very often, but its occurrence strongly suggests that population size is not the only important factor for the reproductive success of rare plants. Our results on *O. purpurea*, in which shading of the populations seemed as important for fruit set as population size (Kos et al., in review), support this view. Light intensity was also important for reproduction in the rare shrub *Gardenia actinocarpa* (Osunkoya-Olusegun, 1999). In the orchid *Calypso bulbosa*, the rate of pollinia export was correlated with the density of the co-flowering shrub *Salix caprea*, but not with population size (Alexandersson and Ågren, 1996).

In *Gentiana cruciata*, competition for pollinators occurred with the abundantly co-flowering *Rubus caesius* (Petanidou et al., 1995). Seed set of the annual *G. uliginosa* was significantly reduced in a plot with a high abundance of *Mentha aquatica*, suggesting that some interspecific competition for pollinators occurred even in this highly autofertile species (Petanidou et al., 1998). Indications for competition for pollinators were also found for the rare orchid *Spiranthes spiralis* (Willems and Lahtinen, 1997). Although most studies on rare plants have focused on the effects of population size and isolation, other studies have demonstrated that resource availability can directly or indirectly affect fecundity and/or seed mass as well (Totland, 1997; Vaughton and Ramsey, 1997; Aizen and Searcy, 1998; Timmerman-Erskine and Boyd, 1999). Fischer and co-workers (1997) found that increased competition under experimentally increased CO₂ had a significant negative effect on seed set of the rare *G. germanica*.

The non-linear relationship between the log of population size and fruit set observed in *O. morio* differed from the linear relationship found in some of the other species. This may be explained by the deceptive strategy of this orchid species, which relies on inexperi-

enced bumblebees that learn how to avoid the species after some time (Nilsson, 1984). Apparently, in *O. morio*, pollinator limitation occurs both in very small (<40 flowering plants) and very large populations (>100,000 flowering plants). In the small populations, this is probably due to the Allee-effect, whereas in the larger populations, the number of bumblebee colonies in the surroundings may become a limiting factor. Similar observations have been done on other deceptive orchids (Ackerman, 1981; Fritz and Nilsson, 1994). The Allee-effects seemed to affect small populations of *O. purpurea* as well, but the remaining populations of this species were probably never so large that problems with pollinator availability could arise. The absence of any similar relationships in *O. militaris* is possibly caused by the fact that it is generally pollinated by solitary bees, which are generally strongly dependent on the availability of nesting sites (Gathmann et al., 1994; Tschamtko and Greiler, 1995). This may cause a higher level of stochasticity than limited population size alone.

Positive relationships between population size and reproductive success have been found in many other plant species (Sih and Baltus, 1987; Jennersten, 1988; Kwak et al., 1991; Lamont et al., 1993; Widén, 1993; Ågren, 1996; Groom, 1998; Gigord et al., 1999; Molano-Flores and Hendrix, 1999). A high plant density is apparently an important component of large population size, and generally is associated with a better seed set and higher outcrossing rates (Van Treuren et al., 1993; Van Treuren et al., 1994; Kunin, 1997; Roll et al., 1997; Ghazoul et al., 1998). In an experimental study on *Brassica kaber* (Kunin, 1997), however, seed set was related to population density but not to population size. This shows that the two variables should preferably be analyzed separately, something that is hard to do in natural populations, unless large numbers of populations can be studied.

Like in *B. kaber*, the correlation between

seed set and population size is absent in several other species (Van Treuren et al., 1993; Alexandersson and Ågren, 1996; Kunin, 1997; Milberg and Bertilsson, 1997; Olfelt et al., 1998; Sawyer and Anderson, 1998; Molano-Flores et al., 1999). Molano-Flores et al. (1999) argued that species that need only small pollen loads on the stigma to initiate seed or fruit set will suffer less from pollen limitation, so that a relationship with population size is less likely. The pollination system will also play a very important role in the relationship. In highly autofertile species, for example, small populations will have similar seed set as large ones. In *Aquilegia canadensis*, a species with a high autofertility, seed set was not related to population size, but the outcrossing rate was low in small populations, suggesting that autogamous selfing occurred more frequently (Routley et al., 1999). The same is probably true for the short-lived gentians *Gentianella germanica* and *G. uliginosa* (Fig. 2). *G. germanica* is at present apparently hardly visited by any insects in its marginal (although occasionally very large) populations in The Netherlands. Since 1992 the frequency of strongly herkogamous plants has decreased in a population that suffers from both pollen limitation and badly-timed mowing, resulting in a higher autofertility (Luijten et al., 1998; Luijten et al., 1999). In *G. uliginosa*, autogamous selfing seems to occur already in very early stages of anthesis, reducing the opportunities for cross-pollination, although bumblebee visits can be frequent in its natural habitat (Petanidou et al., 1998). Still, even in these autofertile short-lived species, pollinators seem more important for reproduction than we expected earlier (Oostermeijer et al., 1998).

Generalist plants, pollinated by various insects groups, have fewer problems with reduced visitation rates in small populations (Waser et al., 1996). However, the self-incompatibility mechanism that is often present in these species may pose an additional problem in small populations, as genetic drift

can reduce the variation in S-alleles and, consequently, the availability of compatible mating types (Byers and Meagher, 1992; Reinartz and Les, 1994; Godt and Hamrick, 1999; Young et al., 1999). In the self-incompatible generalist species studied here, *A. montana*, the availability of compatible mates in small, isolated populations seems indeed more important than pollinator visitation (S.H. Luijten, unpublished data).

IMPLICATIONS FOR THE CONSERVATION OF RARE PLANTS

The answer to the question we posed in this paper is clearly that a large population size is generally important for the successful reproduction of plants. Low reproductive success caused by pollen limitation may occur in many plants (Burd, 1994) but has been reported especially for naturally rare species, often after comparison with a common congener (Karron, 1989; Menges, 1995; Day et al., 1997; Sipes and Wolf, 1997; Willems and Lahtinen, 1997; Bosch et al., 1998; Negron-Ortiz, 1998; Tremblay et al., 1998; Bigger, 1999; Timmerman-Erskine and Boyd, 1999). Yet, there is little or no information on the extent to which this limited production of offspring has consequences for the persistence of local populations, or is in any way one of the causes for the rarity of species (Holderegger, 1996; Byers and Meagher, 1997; Ruiz-De Clavijo, 1997; Witkowski and Lamont, 1997; GIBLIN and Hamilton, 1999). Therefore, it is by no means certain that the observed reductions in reproductive output in small populations of the "new rares" will have any significant impact on their population viability. It is generally assumed that a reduction in the offspring production below the "normal" level will have significant demographic consequences. To our knowledge, only few studies have specifically addressed this question so far (Oostermeijer, 1996; Groom, 1998; Oostermeijer, in press). In the "new rare" *G. pneumonanthe*, a sto-

chastic matrix projection model was made to simulate the population dynamics of the species during heathland succession. Incorporating the relationship between population size and seed production presented in this paper into this model demonstrated that the large reduction in seed set found in small populations hardly had any effect on the simulated population growth rates, and did not increase the probability of extinction (Oostermeijer, 1996; Oostermeijer, in press). However, in this perennial species, the reduced seed set was caused by pollen limitation. This means that the limited number of seeds that was produced was the product of autogamous selfing and thus strongly inbred. Incorporating empirical results on the effects of inbreeding on demographic parameters into the simulation model as well showed that the combination of low seed production and inbreeding depression had a very significant effect on population viability (Oostermeijer, in press). This demonstrates that reduced seed production alone does not necessarily pose a threat to populations of rare plants. The effects of reduced reproduction will most likely be more severe for annuals. In an experiment with the annual *Clarkia concinna*, small populations experienced both chronically lower seed set (due to pollen limitation) and higher extinction rates than larger patches, suggesting that reproduction played a significant role. However, demographic effects of inbreeding were not assessed for this outcrossing species (Groom, 1998).

A high seed production is not only important for local persistence of populations (Groom, 1998), but also for the colonization of new habitats and thus for the survival of regional or even larger-scale metapopulations (Eriksson, 1996a,b; Byers and Meagher, 1997; Ronce and Olivieri, 1997; Groom, 1998). In this context, the results presented and reviewed in this paper are alarming, since there can be no doubt that the reduced seed production in small populations and increased isolation which are associated with

habitat fragmentation will eventually lead to the disintegration of metapopulations and the subsequent local or regional extinction of many plant species.

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