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Effects of fragmentation on pollen and gene flow in insect-pollinated plant populations

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General introduction

Habitat fragmentation and stochastic processes

Human activities often lead to the decline and deterioration of many natural ecosystems. The remaining 'undisturbed' areas are often limited in size and of lower quality than the original ecosystem. These habitat fragments can each sustain only small populations, which are isolated by larger distances, less connecting corridors and more barriers compared to the original situation. It is generally recognized that habitat fragmentation poses a threat to the persistence of many species (Soulé 1986; Andrén 1994; Olesen and Jain 1994; Schemske et al. 1994; Young et al. 1996; Kwak et al. 1998). For birds and mammals, it is estimated that patch size, isolation and the quality of the surroundings seriously influence the persistence of populations, when more than 70% of the original habitat is lost (Andrén 1994).

The organisms in the small and isolated populations, which have survived habitat fragmentation, experience an increase in vulnerability due to stochastic processes (e.g. Barrett and Kohn 1991; Caughley 1994; Schemske et al. 1994). Three different types of stochasticity are distinguished: demographic, environmental and genetic stochasticity. Demographic stochasticity is concerned with random variation in demographic parameters, like birth and death of individuals. One of the most visible cases of environmental stochasticity are catastrophes: sudden extinctions or severe reductions in population size, due to extreme conditions like floodings, volcanic eruptions or epidemic diseases. As Mangel and Tier (1994) have stressed, the occurrence of catastrophes will result in the eventual extinction of any population. For rare species in fragmented habitats, these local population extinctions might well result in complete extinction of the species. The impact of demographic and environmental stochasticity for population extinctions is widely accepted. In contrast, empirical evidence for population extinctions, due to genetic stochasticity, is scarce and its importance for population persistence is possibly much smaller (e.g. Lande 1988; Caughley 1994; May 1995; Bijlsma et al. 1997). Genetic stochasticity is a result of increased random genetic drift and inbreeding in small and isolated populations, which might result in a lower fitness and increased susceptibility to demographic and environmental stochasticity (Van Treuren et al. 1993a; Oostermeijer et al. 1994; Ouborg and Van Treuren 1995; Oostermeijer et al. 1998; Bijlsma et al. 2000). Together, the negative effects of isolation and small population size on fitness are referred to as genetic erosion, which might enhance the extinction risk of small populations on a longer time scale.

Genetic erosion, gene flow and migration

Genetic erosion results from the combined action of random genetic drift and increased mating between relatives in small and isolated populations. Due to random sampling effects, neutral alleles at polymorphic loci have a certain probability to get lost, depending on their initial frequency, q_0 . The probability of loss is $1-q_0$ (Hartl and Clark 1989). Thus, rare alleles get lost by chance more often than common alleles. This occurs, though with lower probability, even when they are beneficial. Loss of alleles will automatically result in a lower frequency of heterozygous genotypes and an increase in homozygosity for common alleles. The resulting decline in overall heterozygosity is often correlated with a lower mean fitness (Frankham 1996). However, fitness is not necessarily always decreased. As long as the common alleles are beneficial in the homozygous state (or less deleterious than rare alleles), increased homozygosity might be advantageous. But even in that case, the loss of alleles will reduce the potential to adapt to future changing environments, implying a risk for long-term persistence (Bijlsma et al. 1997).

In small populations, the frequency of mating between relatives might not only be higher due to an increased average relatedness between individuals. Changes in mating patterns might occur as well, leading to additional (biparental) inbreeding and a further increase in overall homozygosity (Hartl and Clark 1989). Inbred individuals become homozygous for many genes simultaneously, which is generally accompanied by a decrease in fitness, called inbreeding depression (e.g. Schemske and Lande 1985; Charlesworth and Charlesworth 1987b; Van Treuren et al. 1993a; Frankham 1995; Bijlsma et al. 1997; Bijlsma et al. 2000). Together, in small and isolated populations, random genetic drift and inbreeding will result in a loss of alleles, lower genetic variation and increased homozygosity, with usually negative effects on individual fitness and consequently on the mean fitness of the population. The rate at which these processes operate is linearly related to the size of the population, N . The mean heterozygosity decreases with a percentage equal to $1/2N$, due to random genetic drift. The increase in relatedness, represented by the inbreeding coefficient F , is similarly related to the population size according to $\Delta F = 1 / 2N$ (Hartl and Clark 1989). Thus, both random genetic drift and inbreeding are more important in small than in large populations, resulting in a positive correlation between genetic variation (and overall heterozygosity) and population size. Indeed, a positive correlation between population size and genetic variation was reported, for example, for Dutch populations of *Salvia pratensis* and *Scabiosa columbaria* (Van Treuren et al. 1991), *Gentiana pneumonanthe* (Oostermeijer et al. 1994) and several orchid species (Den Nijs et al. 1998). In contrast, Swedish populations of *Scabiosa* and other grassland species did not show such a correlation (Berge et al. 1998; Waldmann and Andersson 1998). A correlation between genetic variation and population size might be obscured by several confounding factors, influencing patterns of genetic variation and heterozygosity. Such factors are, for example, the history of the population, the degree of gene flow between subpopulations, natural selection favouring relatively heterozygous individuals with a higher fitness, and deviations between the censused number of individuals and the effective population size.

The impact of random genetic drift is higher when the effective population size is smaller. Several factors reduce the effective population size below the number of individuals present. Such reductions in effective population size occur after bottlenecks in population size, if only a fraction of all individuals reproduces, when mating is not random, when subsequent generations overlap or other deviations from the assumptions of an ideal population occur (Hartl and Clark 1989). In contrast, gene flow between subpopulations can increase the effective population size, relative to the number of individuals present in the subpopulation. When gene flow occurs, more individuals contribute their genes to the next generation, counteracting the negative fitness consequences of small population size and isolation, thus slowing down the process of genetic erosion. As a rule of thumb, the exchange of one migrating individual per generation should be sufficient to prevent further genetic differentiation between subpopulations (Hartl and Clark 1989; Ellstrand and Elam 1993). Due to gene flow, the effective population size of all connected subpopulations together can be enlarged and genetic erosion may be prevented.

In general, gene flow comprises dispersal of both individuals and gametes. The genetic effects on population structure are comparable, but dispersal of individuals has the additional advantage that it offers an opportunity to escape locally bad conditions and to colonize new habitats (Slatkin 1987; Lesica and Allendorf 1995; Levin 1995; Webb 1998). At the species level, survival under temporally hostile environmental conditions depends on the possibility of successful gene flow. In case of gene flow to previously uninhabited areas, these colonizations result in expansion of the range of occurrence of the species. Given its impact on colonization,

genetic differentiation between subpopulations and effective population sizes, gene flow might be very important for the distribution and persistence of species (Nichols and Hewitt 1994; Neigel 1997; Richards et al. 1999). Indeed, the spatial distribution of scarce vascular plants in Great Britain, for example, was related to their dispersal capacity (Quinn et al. 1994). Species with low dispersal ability showed a high degree of genetic differentiation, due to isolation between subpopulations. Good dispersers generally have a homogeneous population structure, but at the margins of their geographical distribution they may also show genetic differentiation, caused by a high frequency of founder effects. Consequently, intermediate-dispersing species had a less clustered distribution than more extreme dispersal types.

Gene flow in plants

Contrary to animals, most plants can not actively migrate between patches of suitable habitat, due to their sessile life style. Gene flow occurs mainly by means of seeds and pollen grains. Colonization of new habitats and gene flow between existing populations can occur by dispersal of seeds, if followed by successful establishment. Seed dispersal, followed by successful establishment, increases the size of the recipient population and contributes to the exchange of genetic material. Dispersal of pollen grains is only effective if fertilization occurs and seeds are produced. Thus, effective pollen flow only occurs between already existing populations and it has no direct effect on the number of individuals in the recipient population. However, pollen flow might have important consequences for gene flow and the prevention of genetic differentiation between subpopulations.

Dispersal of seeds is a common mode of gene flow for plants. Migration distances of seeds are generally limited, but they can vary up to several kilometers for some plant species. Seed dispersal distances depend on seed characteristics, like size and shape and on the mode of dispersal (e.g. Poschlod et al. 1996; Van Dorp et al. 1996; Kleijn et al. 1997). Heavy seeds, which are dispersed by gravity, typically disperse only over a very short distance, while animals might disperse light or sticky seeds over several kilometers (Fischer et al. 1996). A comparable diversity in transport mechanisms exists for the dispersal of pollen grains. They can be transported by abiotic vectors like wind and water, or by biotic vectors, such as insects, birds or mammals (Real 1983; Bos et al. 1986; Van Dijk 1987; Linhart and Grant 1996; Kearns et al. 1998). Especially for the large group of animal-pollinated plants, the dispersal of pollen is strongly dependent on ecological factors influencing the behaviour of the animals. As for seed dispersal, pollen dispersal distances are usually limited as well, irrespective of the dispersal mechanism. Pollen dispersal distances typically show a leptokurtic distribution (Figure 1), with most dispersal over short distances and incidental dispersal over much larger distances (e.g. Morris et al. 1995; Webb 1998).

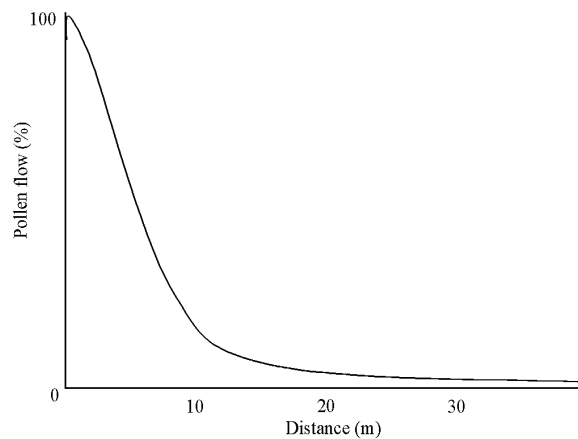


Figure 1. Theoretical leptokurtic distribution of pollen dispersal with distance.

Despite the generally restricted dispersal of seeds and pollen grains, gene flow between subpopulations apparently occurs regularly. Absence of genetic differentiation between plant populations and thus sufficiently high levels of gene flow were frequently reported (e.g. Campbell and Dooley 1992; Krauss 1994; Linhart and Grant 1996; Berge et al. 1998). Several studies distinguished between gene flow by seeds or by pollen, based on the fact that different parts of the plant genome disperse specifically via seeds or pollen grains. In dioecious conifers, gene flow can be measured for maternal, paternal and biparentally inherited genomes separately. Mitochondrial genes are maternally inherited, chloroplast genes paternally and nuclear genes via both parents. In these wind-pollinated species pollen (concerning only paternal genes) dispersed all over the population, but seeds (maternal and paternal genes) dispersed only locally, resulting in different degrees of isolation depending on the type of genome studied (Hu and Ennos 1997; Latta et al. 1998). Differences in the relative importance of seed and pollen dispersal might be expected between wind-pollinated and animal-pollinated plants. Because animal behaviour is thought to be highly local, the relative importance of pollen dispersal for the prevention of genetic differentiation might be expected to be lower in animal-pollinated plants. Pollen dispersal might even be more restricted than seed dispersal. However, pollen flow was found to be less restricted than seed dispersal for several animal-pollinated plants (Krauss 1994; Peakall and Beattie 1996; Berge et al. 1998). These results show that pollen flow can have a substantial influence on patterns of gene flow and the genetic structure of plant populations. Especially in animal-pollinated plants, gene flow by pollen might be highly dependent on ecological factors, influencing the foraging strategy of the pollinators (Richards et al. 1999).

This thesis will focus on the interactions between plants and their animal pollinators and the consequences of these interactions for gene flow by pollen. Attention is directed to changes in pollinator foraging behaviour in response to variation in ecological parameters, which are related to the spatial structure of the plant population. The amount of gene flow between populations, relative to the amount of within-population pollination, is quantified, to assess the opportunity for genetic drift and inbreeding.

Gene flow by pollinators

Dispersal of pollen grains by animal vectors is advantageous for a plant because pollen transfer can be manipulated. If a plant is successful in stimulating its pollinators to fly preferably between plants of the same species (to be 'flower constant'), it can reduce the loss of pollen grains, never reaching conspecific stigmas. Pollinators that switch very often between plant species, are likely to waste most pollen grains on stigmas of a different plant species, resulting in very low pollination efficiency. However, enhancing pollination efficiency might be costly to the plant, because pollinators visit plants for their own benefit. Pollination of the plant is just a side effect of pollinator foraging behaviour, although their foraging strategy is crucial for the pattern of pollen flow experienced by the plant. For this reason, many plants provide pollinators with resources like nectar, pollen, secondary plant metabolites, shelter or mates (Real 1983). Although providing these rewards can be very costly to the plant, it might increase the fidelity of the pollinators and reduce the loss of pollen on stigmas of different plant species (Pyke 1991).

Plants can further influence their pollination efficiency by selecting certain pollinator species. For example, nectar produced in flowers with spurs or deep corollas will be accessible to long-tongued bumblebees only (Comba et al. 1999a). If nectar production is high, the bumblebees will restrict their foraging to such a plant species, resulting in high pollination efficiency. Alternatively, restriction of nectar rewards might result in a shift to other pollinator species, which have different pollen dispersal characteristics. Butterflies and pollen-collecting insects react less strongly to nectar production than (bumble)bees, who need to satisfy both their own high energy demands and those of the individuals in the nests (Herrera 1987; Corbet et al. 1995; Kwak and Velterop 1997). Which pollinator species will be attracted, depends on the type of reward offered by the plant, its quantity and the accessibility to the pollinator (Comba et al. 1999a).

Characteristics of the pollinator species determine the efficiency of pollination and patterns of pollen flow (Table 1, Herrera 1987; Corbet et al. 1995; Harder and Barrett 1996). Removal of pollen from anthers is generally very effective for large and hairy pollinators, like (bumble)bees. Also pollen-collecting insects, which either eat the pollen themselves or feed it to their larvae, are efficient in pollen uptake, although the subsequent deposition of pollen on stigmas might be very low (Conner et al. 1995). Purity of pollen loads is another important measure of pollinator efficiency. Waste of pollen on foreign stigmas is expected to be low for specialist visitors, but the latter are probably rather exceptional (Waser et al. 1996). 'Generalist' pollinator species on the other hand can deposit relatively large amounts of conspecific pollen as well, if they show flower constancy at the level of the individual pollinator. Flower constancy is known to occur regularly for (bumble)bees, but has recently been shown to exist in syrphid flies as well (Goulson and Wright 1998). In contrast, many insects, including butterflies, behave often as generalist foragers, visiting many different plant species (Waser et al. 1996).

Table 1 Differences between specialist and generalist flower visiting insects in some characteristics, important with respect to pollination value. Only general indications are given, in individual pollination systems deviations can occur.

	Specialist	Generalist
Number of individuals	small	large
Visit rate	high	variable, often low
Pollen uptake	high	variable, often low
Pollen deposition	low	high
Flower constancy	high	low
Flight distance	small	variable
Species	bumblebees, honeybees, some butterflies	bumblebees, flies, butterflies

Besides the efficiency of pollen transport, the distance over which pollen flow occurs, is also influenced by the pollinator species. Butterflies usually fly relatively large distances between subsequent flower visits (Schmitt 1980), whereas bumblebees mainly fly between nearest neighbours, because of their high energy demands (e.g. Harder 1988; Keasar et al. 1996; Chittka et al. 1997). Such differences in pollinator flight distances might be translated into different distances of pollen transfer and subsequent variation in patterns of genetic differentiation.

Characteristics as flower constancy, visitation rate and flight distances are not fixed for pollinator species, but can be influenced by the plants. For example, the attractiveness of a plant can be increased by a large floral display (Harder 1988; Goulson et al. 1998; Le Corff et al. 1998; Vaughton and Ramsey 1998). This increased attractiveness might be reflected in a higher number of visits to the plant and more flowers visited per approach (De Jong et al. 1992; Velterop and Kwak 1997). High attractiveness, which increases pollinator visitation rates, might be especially important for very small populations, otherwise overlooked by pollinators (Richards et al. 1999). Alternatively, pollinator behaviour can be influenced by the production of rewards. Larger nectar rewards induced bumblebees to make longer flower visits, to visit more flowers on the same plant and to fly shorter distances between subsequently visited plants (Klinkhamer et al. 1991; Conner et al. 1995; Keasar et al. 1996). Such longer visitation sequences and shorter flight distances increase the frequency of self-pollination and result in locally restricted pollen flow.

Patterns of pollen flow depend on the efficiency of pollen transport and distances over which pollen is exchanged. The type of pollinator clearly influences both aspects of pollen transfer. Also the behaviour of the pollinator is very important and can be modified by characteristics of the plant. It might be expected that pollinator behaviour changes also in response to ecological factors, which are related to the spatial structure of the plant population. The way in which plant population parameters, like size, density and isolation, influence pollinator behaviour, can have profound effects on the resulting patterns of pollen flow.

Measuring pollen flow in animal-pollinated plants

Potentially, pollen flow can contribute considerably to the reduction of genetic differentiation between plant (sub)populations. Therefore, many studies have tried to measure the amount of pollen flow and the pattern of pollen dispersal. In general, pollen flow can be highly variable among species, sites and time periods. Furthermore, it is often difficult to separate gene flow by means of pollen from that by seeds. For these reasons a wide variety of techniques has been developed, each with its own merits and limitations. Frequently used methods range from indirect estimates of overall gene flow, to direct estimates of pollen dispersal distances and inferences about pollen transport based on observations of pollinator behaviour (Slatkin 1985; Slatkin 1987; Neigel 1997).

The amount of effective gene flow can be derived from measures of genetic differentiation between subpopulations. Genetic differentiation is calculated as $F_{st} = 1 - H_s / H_t$, where H_s is the observed heterozygosity, averaged over all subpopulations and H_t the expected heterozygosity, based on overall allele frequencies (Hartl and Clark 1989). The amount of gene flow, more precisely the effective number of migrants, Nm , can be estimated from this F_{st} -value, using the approximation $F_{st} \approx 1 / (4Nm + 1)$ (Hartl and Clark 1989). This method integrates levels of gene flow over longer time scales and includes rarely occurring dispersal over very long distances. In general, empirical studies in predominantly outcrossing plant species show surprisingly low levels of genetic differentiation, indicating the importance of infrequent long distance dispersal (e.g. Campbell and Dooley 1992; Krauss 1994; Linhart and Grant 1996; Berge et al. 1998). However, the pattern of genetic differentiation also includes the effects of processes other than pollen flow, like seed dispersal, colonizations and founder effects, temporary population bottlenecks and differences in selection regimes between populations (e.g. Slatkin 1987; Campbell and Dooley 1992; Nichols and Hewitt 1994; Levin 1995). The impact of such processes is easily confounded with gene flow by pollen, thus other methods have been developed to estimate pollen flow in a more direct way.

When dispersal of pollen grains themselves can be directly tracked, estimates of pollen flow will no longer be affected by seed dispersal or historical events, like colonizations or population bottlenecks. Transport of pollen from different source plants can be distinguished if these pollen grains differ in genetic composition or morphological characters. Alternatively, pollen flow can be estimated from the dispersal pattern of pollen analogues, such as fluorescent dye powders (e.g. Campbell 1985; Waser 1988). By introducing genetic marker alleles, which were previously absent in the experimental area, the exchange of pollen grains between subpopulations was demonstrated over several hundreds of meters (Ellstrand et al. 1989; Skogsmyr 1994). Such distances are often still relatively small, compared to the geographical distances which separate natural (sub)populations. Consequently, natural populations will often be isolated by distances too large to be crossed by pollen grains. Nevertheless, such pollen dispersal between subpopulations occurs over considerably larger distances than commonly found for within-population pollen flow, because of the lack of opportunities for pollen deposition between the subpopulations. Within single populations, extremely limited pollen dispersal distances are reported, independent of the estimation method used. The use of genetic markers, polymorphic pollen grains and pollen analogues all gave estimates of pollen dispersal distances of less than 100m. Due to the leptokurtic distribution of dispersal distances, the majority of dispersal events was even restricted to a few meters from the source plant (e.g. Handel 1983; Thomson and Thomson 1989; Campbell 1991; Pleasants 1991; Manasse 1992; Nilsson et al. 1992; Karron et al. 1995b).

Limited pollen dispersal is a consequence of area-restricted foraging, which is exhibited by many pollinators. Restriction of foraging, within a group of neighbouring plants, maximizes the foraging efficiency of the pollinators by reducing the costs of flying between flowers (e.g. Pyke 1978; Rasheed and Harder 1997). Especially bee species need to optimize their foraging behaviour, because they have to provide nests with food. Since bees are important pollinators for many plant species, patterns of pollen flow are often locally restricted. Butterflies do not provide their progeny with food and their behaviour is much less constrained to visitation of nearest-neighbours. Butterflies regularly skip several plants between subsequent flower visits, but they still fly limited distances (Schmitt 1980; Goulson et al. 1997). Syrphid flies are another important group of pollinating insects (Kearns et al. 1998), but not much is known about their foraging behaviour and flight distances. Although most pollinators generally restrict their foraging within a limited geographical area, many insects are capable of flying much longer distances. Euglossine bees have been reported to fly several kilometers within a single day (Janzen 1971). Bumblebees were observed at a distance of more than 400m from a marking point (Kwak et al. 1998) and butterflies traveled over distances up to 1 km (Hill et al. 1996). Thus, pollinators clearly have the potential to carry pollen over long distances, but apparently they seldom do.

Both, direct observations of pollinator behaviour and studies directed to the dispersal distances of pollen grains are biased in favour of short dispersal distances. Due to practical limitations, these kinds of studies need to focus on the bulk of dispersal events, which occur over short distances (Slatkin 1987). Infrequent dispersal events over extremely long distances are usually beyond the observation limits and easily missed (Slatkin 1985; Campbell 1991; Campbell and Dooley 1992; Neigel 1997). For example, Handel (1982, 1983) studied the dispersal pattern of pollen grains, carrying genetic marker alleles. Because the detection of pollen flow was impossible outside his experimental *Cucumis* populations, his observations of pollen dispersal distances were inherently limited to a maximum of 11m, the size of the experimental plot. Pollen dispersal over the maximal distance did occur regularly, although with a low frequency. Pollen flow was variable between years and sites. In one year, the amount of pollen dispersal showed a gradient in one of the populations, but not in the other, presumably due to the presence of a honeybee hive (Handel 1983). Other studies also report large variation in estimates of pollen flow between years and plots, stressing the need for multiple replicates in order to obtain reliable estimates of pollen dispersal patterns (e.g. Slatkin 1985; Ashman and Stanton 1991; Linhart and Grant 1996; Brody 1997; Goulson et al. 1998).

Pollinator behaviour and pollen flow in fragmented habitats

Pollen flow of animal-pollinated plants depends strongly on local conditions, like the presence of nesting sites and additional food plants for the pollinators. These local circumstances determine to a large extent which pollinator species are available, together with their abundance and foraging behaviour. Due to habitat fragmentation, both the surrounding vegetation, spatial population structure and pollinator species composition will often change, resulting in different patterns of pollen flow (Thomson 1978; Sowing 1989; Conner and Neumeier 1995; Kwak et al. 1998). In specialized pollination systems, habitat fragmentation may lead to the extinction of either the plant or the pollinator species. Extinction of one of the partners will induce the extinction of the second, if other species are unable to take over the ecological function of the former species. Fortunately, most pollination systems are rather general, with plants pollinated by several pollinator species and pollinators foraging on many food plants (Waser et al. 1996).

Nevertheless, a shift in pollinator species composition might still be accompanied by changes in patterns of pollen flow, if the behaviour and pollination efficiency of the new pollinator differ from those of the original species.

Flowering environment

Besides effects of insect species composition, pollinator behaviour depends on the presence of other flowering plants. Habitat fragmentation will frequently result in lower floral diversity and abundance, reducing the diversity and numbers of pollinators that can be sustained by the area (Kwak et al. 1998). As a consequence, negative effects of fragmentation on pollination, seed production and gene flow might happen regularly. Reductions in population size imply an increase in the relative importance of the flowering surroundings, which is often accompanied by increased competition for visitation. The number of pollinator visits to e.g. *Blandfordia grandiflora* was lower when *Banksia serrata* flowered simultaneously (Ramsey 1995). Similarly, the orchid *Spiranthes spiralis* suffered a reduced seed production, due to co-flowering plants (Willems and Lahtinen 1997). In other plant species, more pollen was wasted on co-flowering plants (Murcia and Feinsinger 1996) or seed set declined, due to deposition of heterospecific pollen (e.g. Galen and Gregory 1989; Murphy and Aarssen 1995). Even patterns of pollen flow may depend on the presence of co-flowering plants. Pollen dispersal distances in *Stellaria pubera* were found to be smaller in mixed plots with *Claytonia virginica*, compared to pure stands of *Stellaria* alone (Campbell 1985). The flowering environment can thus have important consequences for patterns of pollen flow in animal-pollinated plant species, although the impact of changes might be difficult to predict (Kwak et al. 1998).

Spatial population structure

Spatial structure includes several different factors, such as the size, shape and density of subpopulations, the degree of clustering within subpopulations and the distance by which subpopulations are separated from each other. These parameters will usually change after habitat fragmentation, which can have a large influence on pollen flow.

Small populations often exhibit a reduction in visitation, reproductive success and genetic variation compared to large and continuous populations (e.g. Jennersten 1988; Sowig 1989; Byers 1995; Oostermeijer et al. 1995). In fragmented natural populations, such negative effects of reduced population size are difficult to separate from similarly deleterious consequences of a simultaneous decline in density. Low plant densities frequently result in a lower number of visits per plant, more flower visits within the same plant, increased selfing rates and reduced seed production (e.g. Ellstrand and Marshall 1985; Dreisig 1995; Karron et al. 1995a). The negative effects of low density on seed set were especially clear when other flowering plant species competed for pollination (Kunin 1993). Several experimental studies suggested that the harmful effects of low density are even stronger than those of small population size (Van Treuren et al. 1993a; Van Treuren et al. 1994; Kunin 1997). A combination of effects of size and (variation in) density might be found for clustered distributions of plants. In tropical trees, almost all trees within a cluster were visited, if the cluster was encountered by a pollinator, inducing a high frequency of local mating (Stacy et al. 1996). Furthermore, small clusters were more easily missed by passing pollinators than large clusters. As a consequence, on average, plants in small clusters received pollen from a larger distance, compared to plants in either large clusters or continuously distributed populations (Stacy et al. 1996). In experimental populations as well, the absence of floral resources in between isolated clumps of plants forced the pollinators to fly

larger distances between subsequent flower visits, compared to continuous distributions. In accordance with the results of Stacy et al. (1996), this resulted in larger absolute distances of pollen dispersal in clumped plant populations (Manasse 1992; Morris 1993). However, at the same time, many pollinators might stay longer within the same group of plants and visit more flowers per plant, thus increasing the frequency of within-patch pollination in relatively isolated groups of plants (Snow et al. 1996; Velterop and Kwak 1997; Richards et al. 1999). In general, an increase in isolation distance is accompanied by a strong reduction in pollen flow. Pollinators usually fly only limited distances and isolation by a few hundred meters already seriously reduced the import of pollen (e.g. Ellstrand and Marshall 1985; Skogsmyr 1994; Goodell et al. 1997; Kwak et al. 1998).

The effects of isolation by distance might be influenced by other aspects of population structure. Large populations at large distances were found to contribute more to pollen import into small populations of wild radish, than nearby small populations (Ellstrand et al. 1989). In *Silene alba*, pollinated by bumblebees and night moths, patch size and isolation distance interacted in a complex manner with each other (Richards et al. 1999). A distance of only 80m severely diminished pollen import. Almost all fertilizations were by plants from the same patch. Extremely small patches received no foreign pollen at all, pollen import into intermediate and large patches occurred rarely with this interpatch distance. In contrast, with isolation distances of 20m, pollen dispersed almost freely and the array of patches could be viewed as a panmictic population (Richards et al. 1999). With such frequent exchange of pollen, the relative size of the target patch compared to its neighbours had an important effect. A larger target patch contributed more to its own pollination success than relatively small patches, resulting in a negative correlation between fertilization by foreign pollen and patch size for these small interpatch distances. Interactions between several components of population structure and flowering environment will often be complex and their consequences therefore difficult to predict. More research is needed to gain insights into these interactions, since habitat fragmentation will regularly induce simultaneous changes in multiple factors influencing gene flow by pollen.

Barriers and corridors

When a habitat is fragmented large populations may become divided in several subpopulations. These subpopulations will often be separated by landscape elements, acting as barriers to pollinator movements. It is well known that large distances act as a barrier, but also roads, waterways, forests, open grasslands etc. might prevent pollinator passage. Butterfly movements were prevented by stretches deciduous woods and mixed forests. In an experimental setup, even a hedgerow of only 1m high drastically reduced the number of butterfly crossings (Fry and Robson 1994). High vegetation structures may hamper dispersal of other pollinator species as well. For example, Westerbergh and Saura (1994) suggested that the presence of spruce forest prevented bumblebee flights between different populations of *Silene dioica*.

Whether certain structural elements in the landscape act as barriers to pollinator movement may strongly depend on the pollinator species, but empirical data are scarce. A study of population structure in *Procllossiana eunomia* revealed that these butterflies did not migrate between different river basins in southern Belgium. Even small geographic distances between river basins were not passed (Nève et al. 1996). Bee species, on the other hand, were able to travel over 1 to 5 km open water (Kwak et al. 1998). Although bees can travel very long distances, male euglossine bees did not cross a forest clearing of only 100m (Powell and Powell 1987). In contrast, similar grassy tracks within a forest were no barrier at all for butterflies, but

even enhanced their dispersal (Sutcliffe and Thomas 1996). Physical barriers can thus have important consequences for the behaviour of pollinators, but virtually no data exist concerning the consequences for pollen flow between groups of plants.

As demonstrated by the forest clearings mentioned before, similar landscape elements can have quite different effects on animal dispersal, depending on the species. Grassy tracks inhibited movement of euglossine bees, but promoted the dispersal of butterflies, thus acting as a corridor for the latter species. The word corridor will here be used for linear stretches of suitable habitat connecting two habitat patches in an otherwise hostile environment. Enhanced dispersal by corridor structures has been reported for several other animal species (Vermeulen 1994; Beier and Noss 1998; Gilbert et al. 1998; Tischendorf et al. 1998). These studies showed that animals might use corridors for dispersal with unknown consequences for the animal population. However, the effects of changes in animal movements on other organisms have mostly been neglected. For example, the influence of corridors on dispersal patterns of pollen grains is largely unknown, although several effects of corridors on pollen flow may be anticipated. A corridor can influence the directionality of pollinator flights, inducing the pollinators to fly along the corridor. Therefore, more pollinators may reach the subpopulation at the other end of the corridor, resulting in increased pollen exchange between the connected subpopulations. In an experiment with *Phyteuma spicatum* ssp. *nigrum*, pollen flow between patches was indeed higher when they were connected by a corridor, compared to isolated patches (Kwak et al. 1998). The short corridor (25m) of linearly arranged *Phyteuma* inflorescences had a beneficial effect on pollen flow, although it had no effect on the visitation rate of the *Phyteuma* patches. Despite longer flight distances along a row of clumped plants, no effect on the mean pollen dispersal distance was found in two other studies (Manasse 1992; Cresswell 1997). The simultaneously observed decrease in flight directionality might have induced the pollinators to stay within the 'corridor' for a longer time. If pollinators get 'trapped' within a corridor, they might lose all their pollen grains on flowers inside the corridor, before reaching the target population at the outer end. Long and attractive 'corridors' might receive more visits and function effectively as barriers to pollen flow, while a short and less attractive corridor might be visited rarely and enhance pollen flow. During visits to flowers in the corridor, pollen of the target species will be lost and probably heterospecific pollen of the corridor species will be picked up. Depending on the flower species in the corridor, this heterospecific pollen might have negative effects on seed production in the target population (e.g. Galen and Gregory 1989; Murphy and Aarssen 1995). A corridor might change flight patterns of the pollinators and result in a loss of conspecific pollen and the uptake of heterospecific pollen on flowers inside the corridor. The combined effects of these changes on pollen flow will strongly depend on the geometry of the corridor and its flower composition. Empirical data concerning pollen transfer along corridors are very scarce.

The research project

Habitat fragmentation has important direct effects on plant reproduction, caused by changes in the quality of the environment and in the demography and genetics of the plant populations. For animal-pollinated plants, additional effects of fragmentation may occur through changes in pollinator behaviour. After fragmentation of a large, continuous population, changes in the species composition of the pollinators and their behaviour may lead to different patterns of gene flow by pollen, with potentially important consequences for the mating system and genetic composition of the plant population (Figure 2). Most research has been concentrated on the effects of reduced population size, reduced density and increased isolation distances between subpopulations on pollen dispersal. In general, these factors have a negative impact on pollen exchange. Furthermore, after fragmentation, the remnant populations often become separated by different types of physical barriers, like roads, railways, agricultural areas or houses. Thus far, the consequences of such barriers on pollen flow are largely unknown. The establishment of corridors has been proposed to overcome the negative effects of geographical isolation, but there is a lack of data concerning their effectivity. Empirical evidence suggests that corridors may be used by animals, but effects on pollen flow are rarely investigated.

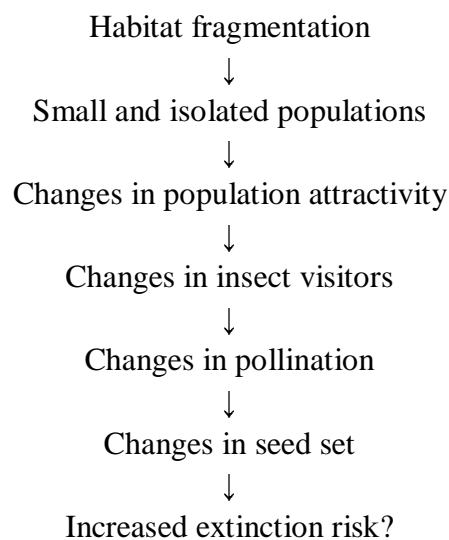


Figure 2. Consequences of habitat fragmentation for insect-pollinated plant populations.

In this project the effects of population fragmentation on gene flow by insect-pollinators in *Scabiosa columbaria* are studied. Different aspects of fragmentation are investigated and their consequences for pollen dispersal between patches are compared. Research is focused on the effects on pollen flow brought about by changes in insect behaviour, in response to variation in spatial population parameters, like isolation distance, physical barriers and corridors. *Scabiosa columbaria* is pollinated by various insect species, which differ in pollination efficiency and reaction to population fragmentation. Special attention is given to the foraging behaviour and pollination efficiency of syrphid flies. Information about this group of insects with respect to pollen flow is scarce, despite their important contribution to the pollination of many plant species, especially in small populations or at low flower densities (Kunin 1993; Conner and Neumeier 1995; Kwak and Velterop 1997; Kearns et al. 1998). Empirically, the interactions between plant population parameters and the behaviour of the pollinating insects are studied. The impact of these interactions for the frequency of within-patch fertilizations and gene flow by

pollen is investigated. Because different types of pollinators react differently to variation in plant (population) characteristics, the effects of habitat fragmentation may vary between pollination systems. However, comparisons between pollination systems are difficult to address experimentally. The relation between type of pollinator species and the resulting vulnerability of the plant population, after fragmentation of its habitat, is therefore explored theoretically.

Plant species involved in this study

Experiments are performed using *Scabiosa columbaria* L. (Small scabious, Dipsacaceae). *Scabiosa columbaria* is a perennial species occurring in calcareous grasslands, and is rare in The Netherlands. The plant flowers from the end of July till October. The small, tubular, blue-violet flowers are arranged in heads (40-100 flowers per head) and contain a single ovule each. Each flower starts male flowering for about 2 days, followed by a neuter phase of varying length. After all flowers in a head have passed the male phase, the whole head is female for a single day. Although the plant is self-compatible, it is predominantly outcrossing (Van Treuren et al. 1994). Both anthers and stigmas protrude out of the flower and are easily touched by various species of insects. *Scabiosa columbaria* can be visited by a wide variety of insects. In the small and isolated Dutch populations, its main pollinators are syrphid flies and bumblebees (Kwak and Velterop 1997). In France, on the other hand, large populations of *S. columbaria* can still be found, which are pollinated by a specialist solitary bee species (*Dasygaster argentata*), honeybees and butterflies.

Scabiosa columbaria experienced a severe population fragmentation during the last few decades. The number of populations in The Netherlands declined with more than 50% (Ouborg 1993a). With only two exceptions, the remaining populations are small, with less than 500 flowering individuals (often less than 100). Although variation in fitness related characters was unrelated to population size, small populations showed a lower genetic variation than large populations (Van Treuren et al. 1991; Van Treuren et al. 1993a). In view of the strong inbreeding depression observed in experiments, there is a clear potential for genetic erosion in this species. The degree of inbreeding (depression) and local mating is largely determined by the behaviour of the pollinating insects, because seeds are predominantly dispersed by wind within a few centimeters from the maternal plant (Verkaar et al. 1983). In an earlier study, the insects were shown to react strongly on plant density (Van Treuren et al. 1994). In patches with low plant densities, pollinators induced a high frequency of self-pollination. This was not reflected in a high frequency of seeds originating from selfing, because high inbreeding depression prevented the development of these seeds. Therefore, patches with low plant densities were still highly outcrossing, but at the cost of severe reduction in seed set (Van Treuren et al. 1994). In fragmented populations of *S. columbaria*, changes in pollinator behaviour may have important consequences for patterns of gene flow and/or plant reproduction.

Outline of the thesis

The research project aims to gain insight in the effects of habitat fragmentation on the genetic composition of plant populations and the role of gene flow in this process. In many insect-pollinated plant species, gene flow is strongly dependent on pollen dispersal, which in turn depends on the foraging behaviour of the insect visitors. Therefore, the thesis focuses on the impact of insect-pollination on gene flow by pollen.

This **first chapter** gives an introduction to habitat fragmentation, the concept of genetic erosion and the importance of gene flow. Special attention is given to gene flow by pollen and several methods are introduced to estimate pollen dispersal distances. Changes in pollinator behaviour in response to different aspects of habitat fragmentation and the consequences for pollen flow are discussed. Finally, this chapter introduces *S. columbaria* as the experimental plant species and gives an outline of the thesis.

The **second chapter** concentrates on characteristics of the pollinators. *Scabiosa columbaria* can be visited by many insect species, like bumblebees, honeybees, syrphid flies and butterflies. Additionally, populations of *S. columbaria* in France are sufficiently large to sustain a specialist bee species, *Dasygaster argentata*. For all visiting species, multiple aspects of pollination efficiency are measured, such as flower constancy, foraging speed, body load of pollen and deposition rate. The consequences of differences in these pollinator characteristics for the amount, composition and dispersal distance of pollen are studied.

Patterns of gene flow by pollen are assessed in a fragmented experimental population. A linearly shaped population of *S. columbaria* is created in a meadow in Assen, The Netherlands. This experimental population consists of several, equally sized patches and is pollinated by naturally occurring insects. Under these semi-natural conditions, insect visitation to the patches is observed and patterns of pollen flow are determined in relation to different aspects of population fragmentation. First, multiple methods to estimate gene flow by pollen are compared (**chapter 3**). Dispersal of pollen grains, pollen analogues (fluorescent dye powder) and allozyme alleles is simultaneously observed. Additionally, insect species composition is determined and some observations are made on insect behaviour. Patterns of pollen dispersal and gene flow in this reference experiment can then be used to evaluate the consequences of population fragmentation on the effective gene flow by pollen between patches of *S. columbaria*.

The relative impact of isolation by distance and the effects of physical barriers are the focus of **chapter 4**. Isolation by distance is achieved by increasing the distances between patches from 25m to 100m and 200m. The influence of barriers, like hedgerows, is simulated by using camouflage nets. Visual isolation is obtained by putting a camouflage net in between two of the three patches, separating a single patch from the others. Insect behaviour and pollen flow in response to these different types of isolation are compared.

In **chapter 5**, the potential of corridors to promote pollen flow between patches is investigated. Since quality and arrangement of the corridor may be very important for its effect on pollen dispersal, different types of corridors are used to connect patches. Corridor geometry can be either linear or perpendicular to the axis connecting the patches and plants can be spaced evenly or clumped. The flower composition of the corridor is varied in linearly arranged corridors, with an even spacing between plants. Insect behaviour along the corridor is observed and the resulting pollen dispersal is studied. Dispersal of conspecific and heterospecific pollen is measured along the corridor and in the patches connected by it. Both size and composition of the stigmatic pollen loads are evaluated in relation to the composition and orientation of the corridor.

Different pollinator species react differently to fragmentation of the plant population. As a consequence, the vulnerability of the plant population to negative effects of habitat fragmentation may depend on its pollinator species. In **chapter 6**, the impact of variation in the pollinator type for genetic erosion after population fragmentation is investigated theoretically. Since in large populations adaptation of plant and pollinator may be expected, the optimal architecture of the plant, in relation to its main pollinator type, is determined. Starting with a large and adapted plant population, habitat fragmentation is then simulated as a reduction in population size, an increase in isolation between subpopulations or a shift in pollinator species. The effects of fragmentation on the viability and genetic composition of the plant population are determined. The impact of pollinator type on the vulnerability of the plants is evaluated.

Finally, an overview of the results is given in the summary. All experiments described in chapter 3 to 5, are performed with a similar setup, using patches with and without treatment. The effects of increased distances, physical barriers and corridors can thus be expressed relative to an isolation distance of 25m. Using the results of chapter 3, the changes in pollen dispersal patterns are extended to their effects on the mating system and genetic structure of the plant population. The impact of several aspects of habitat fragmentation on pollen flow and its genetic consequences is evaluated.