Stellingen

1. Voor de beoordeling van potentiële barrières in het landschap voor de genetische uitwisseling tussen plantenpopulaties is de menselijke waarneming vaak niet maatgevend en kan daardoor gemakkelijk tot onjuiste conclusies leiden.
   (Dit proefschrift, hoofdstuk 4)

2. Net als op de beurs geldt voor de keuze tussen bestuivingssystemen: grote risico's kunnen leiden tot grote successen maar ook tot grote verliezen.

3. Als gevolg van hun gevoeligheid voor toevalseffecten en specifieke omstandigheden, noodzaakt het onderzoek naar kleine populaties tot grotere steekproeven, die echter moeilijk te verkrijgen zijn voor zeldzame soorten.

4. Wellicht zou de mens autowegen moeten ervaren zoals de meeste organismen corridors: beide moeten dan gewaardeerd worden als leefgebied en niet uitsluitend als verplaatsingsroute.

5. Ook voor zweefvliegen geldt: onbekend maakt onbemind maar niet onbelangrijk.
   (Dit proefschrift)

6. Het gebruik van de zinsneden ‘it is obvious’ of ‘it is easy to see’ in theoretische artikelen dient meestal slechts om te verhullen dat de uitleg te ingewikkeld is om in een kort bestek uiteen te zetten.

7. Het Nederlandse systeem van academische titulatuur, waarbij vier jaar gewerkt moet worden om een ‘s’ te verliezen, leidt niet alleen tot veel verwarring onder buitenlandse wetenschappers maar ook onder niet-academici in Nederland.

8. De eisen aan de diplomatieke vaardigheden van promovendi stijgen exponentieel met de toename van het aantal begeleiders.

9. De algemene opvatting dat zwangerschap van vrouwelijke werknemers nadelige gevolgen heeft voor de arbeidsproductiviteit, wordt door dit proefschrift weerlegd.

10. Vroeger werd geleerd dat klikken stout was, maar met het toenemend gebruik van Windows blijkt dubbelklikken maatschappelijk wel aanvaardbaar.

RIJKSUNIVERSITEIT GRONINGEN

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

Proefschrift

ter verkrijging van het doctoraat in de
Wiskunde en Natuurwetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus, dr. D.F.J. Bosscher
in het openbaar te verdedigen op
maandag 19 juni 2000
om 14.15 uur

door

Odilia Velterop

geboren op 24 september 1970
te Amsterdam

Promotores:	Prof. Dr. W. van Delden Prof. Dr. J. van Andel
Co-promotores:	Dr. R. Bijlsma Dr. F.J. Weissing
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Contents

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Voorwoord

Nu dan eindelijk, het laatste loodje is het voorwoord. Veel later dan ik bedoeld had toen ik begon aan mijn project. De start was gemakkelijk: vol enthousiasme en goede moed veldwerk doen, honderden analyses in het allozymlab en bezig met theoretische modellen. De praktijk bleek echter weerbarstig en het viel niet mee om alle onderdelen evenveel aandacht te geven. Ik heb moeten leren om zelf keuzes te maken, cruciaal in een dergelijk breed onderzoeksgebied en met zo veel begeleiders. Net als het omgaan met de grote werkdruk bij de begeleiders en de noodzaak om tijd te claimen. Niet bepaald mijn stijl en het heeft me dan ook veel motivatie en tijd gekost. En hoewel ook promoveren relatief is, ben ik heel blij dat de spreekwoordelijke laatste strohalm sterk genoeg bleek om de eindstreep te halen.
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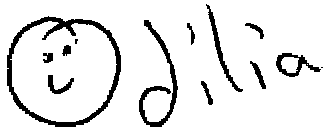

General introduction

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).

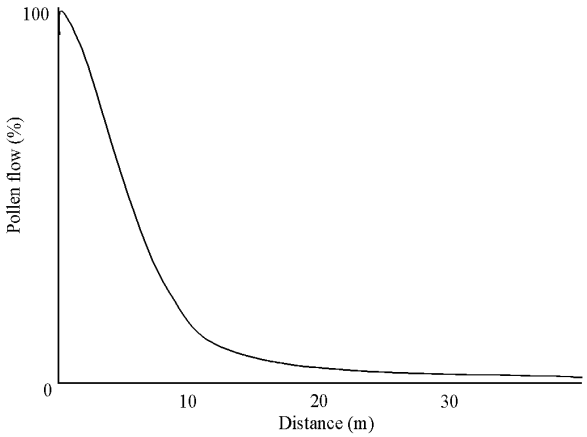

Figure 1. Theoretical leptokurtic distribution of pollen dispersal with distance.

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 attractivity 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 attractivity 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 attractivity, 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; Sowig 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 *Proclossiana 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 loose 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.

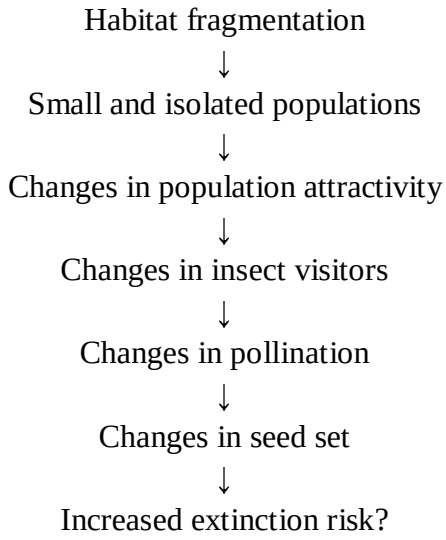

Figure 2. Consequences of habitat fragmentation for insect-pollinated plant populations.

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 (*Dasypoda 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, *Dasypoda 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.

Pollination value of insect visitors of *Scabiosa columbaria* (Dipsacaceae)

With M.M. Kwak and J. van Andel

Summary

The pollination value of insect visitors (small flies, syrphids, bumblebees, honeybees, moths and butterflies) of the Small scabious (*Scabiosa columbaria*) in Dutch and French populations was recorded. Several foraging characteristics, like the number of heads (capitula) visited per minute, flight distances, preference for male or female heads, residence time per head, pollen deposition per head and purity of the body loads carried by insects were analysed. Syrphids and bumblebees, frequent visitors of the Dutch populations, were comparable in pollination value concerning the number of heads visited per minute, purity of the body load and flight distances. However, bumblebees deposited a larger number of *S. columbaria* pollen grains/stigma/second than syrphids. The diversity of the body load of both bumblebees and syrphids was high; pollen from other plant species found in these loads were: *Origanum vulgare, Daucus carota*, *Centaurea* spec. and Compositae. Small flies were poor pollinators due to a long residence time and low deposition rate. Honeybees, frequent visitors in French populations, were good pollinators concerning their pollen deposition on stigmas and the high purity of the body load but their foraging speed was moderate. The specialistic bee species *Dasypoda argentata* was observed only in French populations. Specifically the females, scored high for all behavioural characteristics related to pollination. Butterflies, present in both Dutch and French populations, were poor pollinators but had large flight distances compared to all other insect species and are important for long-distance dispersal. Although some insect species showed a preference for male or female heads this did not lead to an exclusive visitation of one of the sexes. Almost all taxa contributed to the pollination of *S. columbaria* but they differed in various aspects of both the quantity (number of pollen deposited) and the quality (purity of pollen depositions and chance of cross pollination), emphasizing the importance of the presence of a diverse visitor guild for a plant species.

Introduction

Many of the most elaborate examples of coevolution can be found in pollination systems (Anstett et al. 1997). However, plant species pollinated by a single pollinator are far less common than plant species pollinated by more than one taxon. In general, pollination systems often are more generalized and dynamic (Ollerton 1996; Waser et al. 1996). Plant species may be visited by a variety of insect species, belonging to various taxa (Herrera 1989; Olesen and Warncke 1989a; Kato 1996; Comba et al. 1999b; Memmott 1999) and this species composition may vary annually, seasonally, among and within populations and from hour to hour (Herrera 1990, 1995, 1996; Ashman and Stanton 1991; Eckhart 1995; Ramsey 1995; Fishbein and Venable 1996; Traveset and Sàez 1997; Comba et al. 1999b). For example, Memmott (1999) found that plant species in a meadow in Great Britain were visited by a median of seven species of insects (range 0-48) and insects visited a median of three species of plants (range 1-18).

Not all visitors are pollinators and visitor species may differ in their efficiency as pollinators. Visitors differ for instance in body size, abundance, period of activity, foraging speed and flight distances and this may result in differences in pollen deposition. The pollination value can be defined both quantitatively (number of visits and number of conspecific pollen grains deposited) and qualitatively (origin of pollen, self, cross and heterospecific pollen).

Crucial for the pollination value of an insect species is the possibility of contact with anthers and stigma. Variation occurs in the duration of visit per flower and the size and composition of the pollen load carried and all these differences influence the pollination value of a particular insect species (Waser 1982; Schemske and Horvitz 1984; Sugden 1986; Herrera 1987, 1989; Ramsey 1988; Waser and Price 1990; Dieringer 1992; Kwak 1993; Fishbein and Venable 1996; Kwak and Velterop 1997; Traveset and Sàez 1997). In addition, various visitors may have different effects on the male and female reproduction (Stanton et al. 1991). Pollen collecting bees are good in pollen removal but poor in pollen deposition, thus from the plant's point of view a waste of pollen occurs (Wilson and Thomson 1991).

Flowers can be visited by generalists and specialists. Specialized bees may remove more pollen per time than generalists do (Strickler 1979; Cane and Payne 1988) but this does not necessarily mean that they are good pollinators. Specialists are thought to carry pure pollen loads, consequently making conspecific pollen depositions, but analysis of pollen in the scopae of various oligolectic bee species showed significant amounts of foreign pollen; in most cases a pollen shortage for the bees was clearly evident or indicated by climatological data (Cruden 1972). However, the pollination value of oligo- and monolectic bees (specialists) compared to that of polylectic species (generalists) on the same plant species is largely unknown. Both Ashman and Stanton (1991) and Cane and Payne (1988) found that the specialist bee was the best pollinator of the studied species. However, seed set in *Claytonia virginica* did not differ after a visit of a specialist (*Andrena erigeniae*) or generalist insect species (*Bombylius major*) (Motten et al. 1981). Also Olsen (1997) found that the specialist bee species was not a better pollinator than other visitors were.

This chapter presents data on the pollination value of insect species visiting *Scabiosa columbaria* populations in The Netherlands and France. We compare both quantity and quality aspects of the pollination value of generalist and specialist insect species. This chapter is part of a larger project in which the consequences of population fragmentation for pollen and gene flow by insects is studied.

Material and methods

Plant species

Scabiosa columbaria L. (Small scabious, Dipsacaceae) is a perennial, outbreeding species, occurring on dry grassy places on calcareous soils. The main flowering season starts at the end of July and continues till the end of September in undisturbed situations (plants are often grazed or mown but regrowth is possible). The plant forms one to ten flower branches, with 1-20 heads (capitula) each. The bluish-lilac flowers (3 mm long) are arranged in hemispheric heads (diameter 1-3.5 cm), with on average 30-100 flowers per head. The first flowers are displayed in two crowns at the edge and near the center of the head. Outer flowers have 3 enlarged petals. The flowers are protandrous. In all phases flowers contain nectar.

The populations

In The Netherlands *S. columbaria* is a rare species, generally occurring in small populations and only in a few large populations (Ouborg 1993b). Investigated Dutch populations (Wrakelberg, Kruisberg, Wijlre), are situated in verges or nature reserves in the southern part of The Netherlands. Observations were also made in two artificial populations in the northern part of The Netherlands: Haren and Assen. Not all observations could be made in the same population due to practical reasons. In France, extensive areas with *S. columbaria* can be found in abandoned agricultural areas (Departement Savoie). Populations sampled were Modane, Bramans cave, Bramans plateau and Bramans Transformer house. Also a population in the North of France (near Colombey-les-Belles, Departement Meurthe-et-Moselle) in a chalk grassland and along an abandoned railway was sampled.

Observations

1. Flower development

The anthesis of heads was observed by following the development of eight heads, starting on 11 September 1996 in the Assen population. Each evening the newly opened flowers were counted and painted and the moment of stigma receptivity noted. The development of anthers was also observed by checking marked flowers every half an hour.

2. Insect activity during the day and behaviour

Activity during the day and foraging characteristics were measured for the most frequent insect species. Data for each characteristic were collected within two hours on the same day for each species because variation between times, days and years in the various measurements may overrule the differences between insect species.

To measure activity during the day (between 8.30-18.30 h) insects were counted every half an hour in transects or in permanent plots during 10 minutes during sunny weather. This was done in the Dutch population Wrakelberg where syrphids and butterflies were frequent visitors and in two French populations (Bramans cave and Bramans plateau) with large numbers of the specialist bee species *Dasypoda argentata* (oligolectic on *S. columbaria*, Westrich 1990). Behaviour of insects was described and photographs were made (see Plate 1).

Plate 1. Insect visitors on heads of *Scabiosa columbaria*. A. *Siphona geniculata*, a small fly species, on a head in the male phase, B. *Eristalis tenax*, a syrphid species on a female heed, note the *S. columbaria* grains on the head, C. *Eristalis intricarius*, a very hairy syrphid species on a male head, D. the moth *Autographa gamma*, note the introduced tongue and the pollen on the legs, E. *Bombus pascuorum*, a bumblebee visiting a male head, note the pollen adhering to the pile, F. *Dasypoda argentata*, a female of a specialistic bee species visiting a male head. Note the position of the wings typical for an individual foraging for pollen and note the pollen grains between the long hairs of the hind legs.

3. Flight distances

Flight distances between subsequent flower visits are often used to estimate pollen flow distances (large distance corresponds to more cross-pollination, a quality aspect of pollination). However, due to pollen carry-over flight distances often give an underestimation of distances of pollen flow. Therefore, flight distances were measured in two ways: as the distance between two successive flower visits and as the overall distance, measured as a straight line, between the first and eleventh visited head. The first method was used in the large Dutch population Wrakelberg and in the large population Bramans cave in France; the second method only in the population Bramans cave.

4. Foraging speed

Foraging speed is measured as the number of heads visited per unit time, assuming that a high foraging speed promotes cross-pollination. Insects foraging on *S. columbaria* were followed during their visits to 10 heads and the total time, including flight time was recorded. Data were collected in the Dutch population Assen and the French population Bramans cave.

5. Residence time

Residence time per head (time spent on a head with active foraging behaviour) is a measure for the quantitative aspect of the pollination value. A long stay may result in the uptake of more pollen on a male head and in the deposition of more pollen on a female head. The residence time per head was measured as seconds spent per head for various insect species in the Dutch population Assen and the French population Bramans cave.

6. Sex preference

A strong preference for either male or female heads may result in (very) poor pollination. Preference for female or male heads of individual insects was measured by counting the number of insect visits to female and male heads in an area with a known number of female and male heads present. Observations were made during the morning (11.00-12.00 h) and during the afternoon (15.30-16.30 h) in the Dutch population Assen and the French population Bramans plateau.

7. Pollen loads on insect bodies

The presence and size of the pollen load on the bodies of insects is an indication of their potential value as pollinator. Pollen loads on the bodies of insects visiting *S. columbaria* were collected between 12.00 and 16.00 h when nearly all insect species were actively foraging. The ventral side of the body, the side that potentially made contact with the stigma, was cleaned with a piece of sticky gel and a microscope slide of this gel was prepared (Beattie 1972). If possible, 10 individuals per species and per population were sampled. Total number of pollen and the number of pollen were counted. A reference collection of pollen of plant species flowering in the same area was prepared by depositing pollen from an anther directly on the gel.

8. Pollen deposition

Pollen deposition on stigmas of virgin female heads was determined by allowing an insect, foraging in a *S. columbaria* population, to visit the head. Residence time per head was measured too. After it had left the head, the insect was kept in a cage to prevent resampling. The number of pollen grains deposited per stigma was counted using a 20x hand lens (size of *S. columbaria* grains 50-70 µm, pers. comm. G. Romeijn) and pollen deposition per stigma per second was calculated. Residence times per virgin head appeared to be very variable, even within insect species (Table 6, range 4.4-77.1 seconds, Table 7, range 0.4-30.1 seconds). Therefore, the same procedure was followed but visits were terminated after 20 seconds (only for Dutch species). Pollen deposition per stigma per second was calculated.

Pollen deposition by small flies could not be measured in the way mentioned above. Their pollen deposition was measured in cages (two cages with each 20 flies) with one male and six virgin female heads at the start of the experiment. After three hours of visitation the number of pollen deposited on the stigmas was counted. As comparison, the pollen deposition of five *Eristalis tenax* individuals in a cage was measured similarly.

Results

1. Flower head development

Scabiosa columbaria heads started to flower with a few flowers (2-6) per head, the next day often flowers of the second crown opened. Flowers first presented four anthers, in a sequence of two groups of two. The development of stamens was very rapid: once the anthers emerged from the flower, they opened within an hour, presenting via a longitudinal split the pollen grains (on average 340 grains per anther, pers. comm. G. Romeijn). The anthers could change in position during the anthesis from vertical to a more horizontal position; they were connected to the stamens and could change in position also as a result of the touch of insects. Within a few hours the anthers fell off, in the field often stimulated by frequent visitation. After an individual flower had been male, it entered a neutral phase of varying length till all flowers within the head had been male. All flowers became female at the same day and could be pollinated. Generally, the female phase took only a single day. Absence of pollination increased the duration of the female phase. Both anthers and styles protruded out of the small, tubular flowers and could easily be touched by visiting insects.

The increase in percentage of open flowers per head during the period was nearly linear (Figure 1), measured in the Dutch population Assen. Heads with a large number of flowers (>65 flowers) did not have a longer flowering period than smaller ones (<65 flowers per head). All heads became female after 7-9 days of flowering.

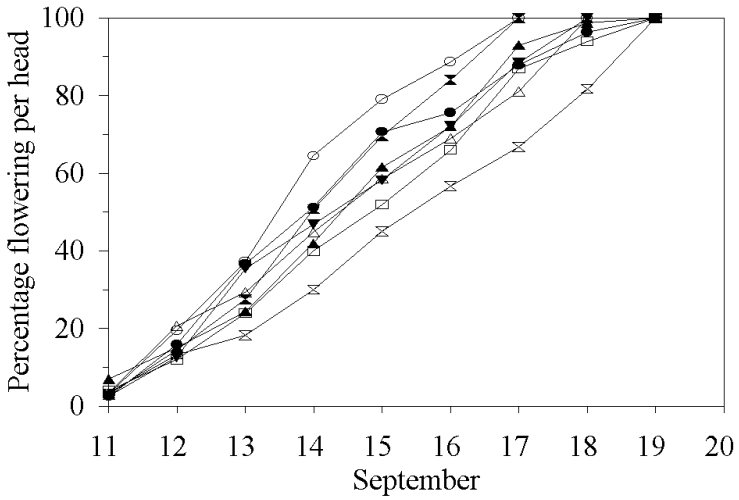

Figure 1. The development of individual heads of *Scabiosa columbaria* in 1996. Open symbols: heads containing less than 65 flowers; filled symbols: heads containing more than 65 flowers.

The marked protandry of heads, with virtually non-overlapping male and female phases (flowers entered a neuter phase after the male phase) resulted in a low chance of spontaneous selfing. However, sexual phases of heads were not synchronous within a plant so geitonogamy via insect visitors was possible. Due to the difference in duration between the male and female phase, the percentage female heads in a population could vary between 5 and 30.

2. Insect activity during the day and behaviour

Insect activity increased rapidly after 8.30 h in the morning (Figure 2). In the Dutch population Wrakelberg, the number of insects remained rather stable between 12.00 and 16.00 h (Figure 2A). In the French populations Bramans cave and Bramans plateau, both *Apis mellifera* and *D. argentata* increased rapidly in numbers during the morning hours, fluctuating at a high level after 11.30 h (Figure 2B, C). After 18.30 h, activity of the insects decreased rapidly.

Frequently visiting insects belonged to Diptera, Hymenoptera and Lepidoptera. Especially Syrphidae with *Eristalis tenax*, *Eristalis arbustorum* and *Helophilus pendulus* and the small fly *Siphona geniculata*, belonging to Tachinidae were frequent visitors. Among Hymenoptera bee species *Bombus* spec., *Psithyrus* spec., *A. mellifera*, *D. argentata*, *Coelioxys rufescens* and the wasp species *Bembix rostrata* were frequent visitors. Lepidoptera, both butterflies as *Inachis io*, *Aglais urticae*, *Pieris rapae*, *Melanargia galathea* and day-flying moths as *Autographa gamma*, *Zygaena filipendulae* and *Zygaena trifolii* were observed regularly.

The foraging day of bumblebees was rather long: starting as the first visitors and continuing as the last ones. Most frequent syrphid species were active during the day between 10.00 and 16.00 h. A syrphid species as *Syrphus balteatus* was mainly active early in the morning and then disappeared. Moths in the Dutch population Wrakelberg had their peak occurrence around 11.00 h but remained present during the whole day.

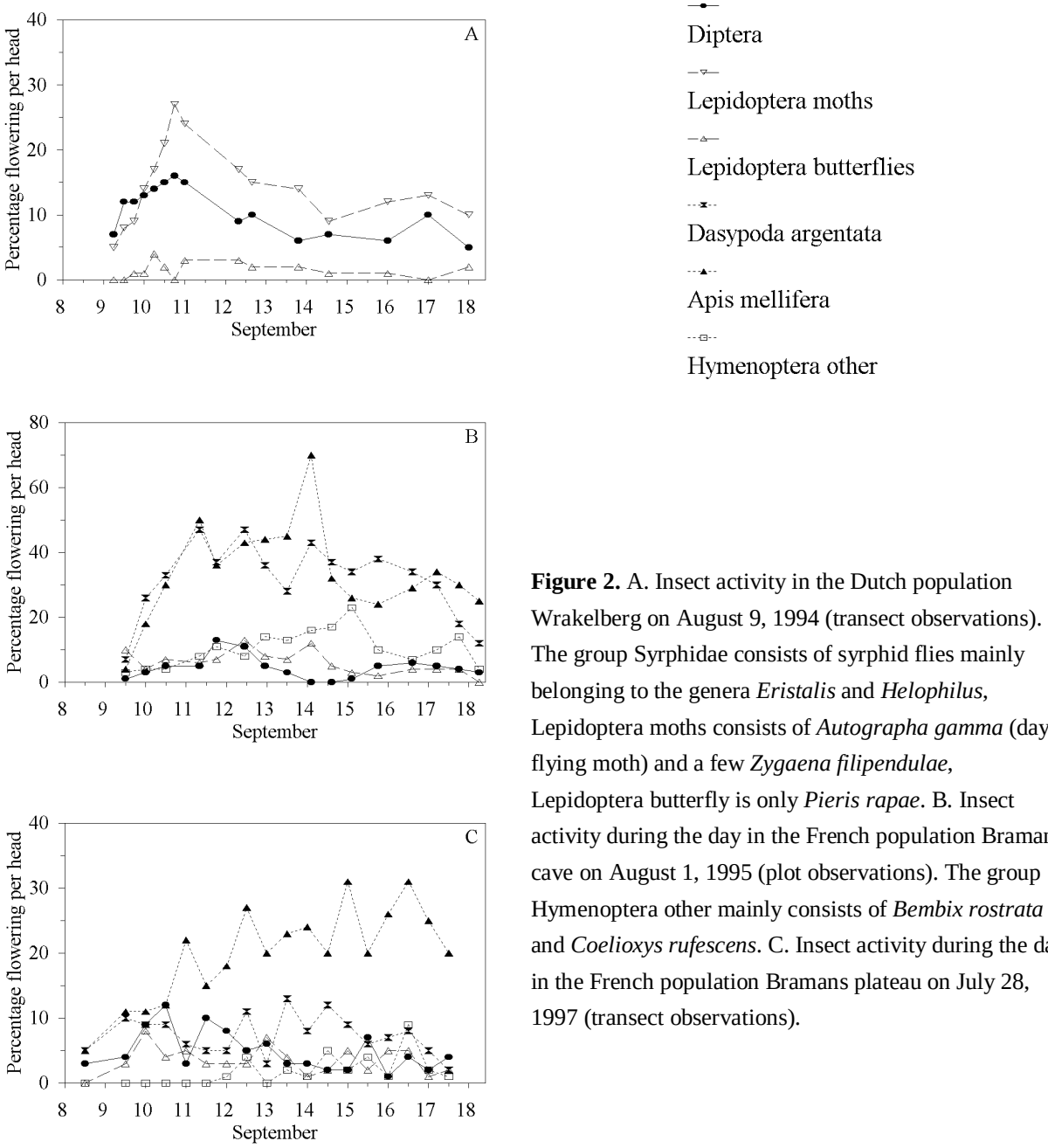

Figure 2. A. Insect activity in the Dutch population Wrakelberg on August 9, 1994 (transect observations). The group Syrphidae consists of syrphid flies mainly belonging to the genera *Eristalis* and *Helophilus*, Lepidoptera moths consists of *Autographa gamma* (day-flying moth) and a few *Zygaena filipendulae*, Lepidoptera butterfly is only *Pieris rapae*. B. Insect activity during the day in the French population Bramans cave on August 1, 1995 (plot observations). The group Hymenoptera other mainly consists of *Bembix rostrata* and *Coelioxys rufescens*. C. Insect activity during the day in the French population Bramans plateau on July 28, 1997 (transect observations).

Insects picked up pollen from the anthers and deposited them on female heads while foraging for nectar and/or pollen (see Plate 1). Most insect species foraged for nectar, introducing their tongue in the small tubular flowers. In the Dutch populations, the syrphid species *S. balteatus* foraged for pollen early in the morning, as soon as the anthers opened. This species inspected female heads for their sex during a very short "touch". Some individuals of other syrphid species ate pollen directly from anthers or stigmas. During foraging, syrphids frequently paused to clean their face and proboscis and they consumed the pollen thus collected.

The pollen-collecting females of the specialist bee species *D. argentata* also touched female heads very shortly (<1 second) especially during the morning. On male heads they rapidly moved while their wings were hold side way and upwards without introducing their tongue into the flowers (see Plate 1). Nectar-collecting females however, spent more seconds per female or male head (see residence time) and introduced their tongue into the flowers while the wings were in a horizontal position, more or less folded over each other. Males of *D. argentata* visited *S. columbaria* heads for nectar but they also hunted for mating. During this search for females they flew large distances. They touched females that visited *S. columbaria* heads and so they disturbed flower visitation of these females. Males slept in the field hanging below the heads, females disappeared, probably into their nests. Nests of *D. argentata* within the *S. columbaria* population were found in the ground.

Generally, in the Dutch populations Syrphidae were the most common visitors and in some years reasonable numbers of bumblebees, Lepidoptera and *S. geniculata* occurred. In the French populations Lepidoptera, and the species *A. mellifera* and *D. argentata* were the most frequent visitors. Foraging characteristics were measured for these taxa.

3. Flight distances

Flight distances of insect visitors foraging on *S. columbaria* differed markedly (Figure 3). In the Dutch population Wrakelberg the species *E. tenax* and *A. gamma* flew short distances: more than 90% of the measured distances were shorter than 2 meter. For both species flights over distances larger than 10 meter were never observed. In contrast, the butterfly *P. rapae* made 15% of its flights over distances larger than 10 meter with some flights over more than 100 meter (Figure 3A). In the French population, all Hymenoptera species, including the wasp species *Bembix rostrata*, flew short distances, less than 2 meter; nearly all distances were shorter than 1 m. In contrast, 61.3% of the distances flown by Lepidoptera was >2 meter (Figure 3B).

Foraging distance, measured as the overall distance between the first and eleventh visited head, differed between *D. argentata* and *A. mellifera* (Figure 4). Males of *D. argentata* flew significantly larger distances (270 ± 32 cm, mean ± s.e.) than did females of *D. argentata* (124 ± 9 cm) and *A. mellifera* (149 ± 17 cm) (Kruskal-Wallis test, $P<0.005$). The frequency distribution (Figure 4) also shows that *A. mellifera* had an intermediate position between *D. argentata* males and females. For males 32.2% of the flown distances were □3 meter, for females this was only 2.3% and for *A. mellifera* 9.2%.

In summary, an increase in distances between two visits to heads of *S. columbaria* was found in the order of Hymenoptera, Syrphidae and Lepidoptera.

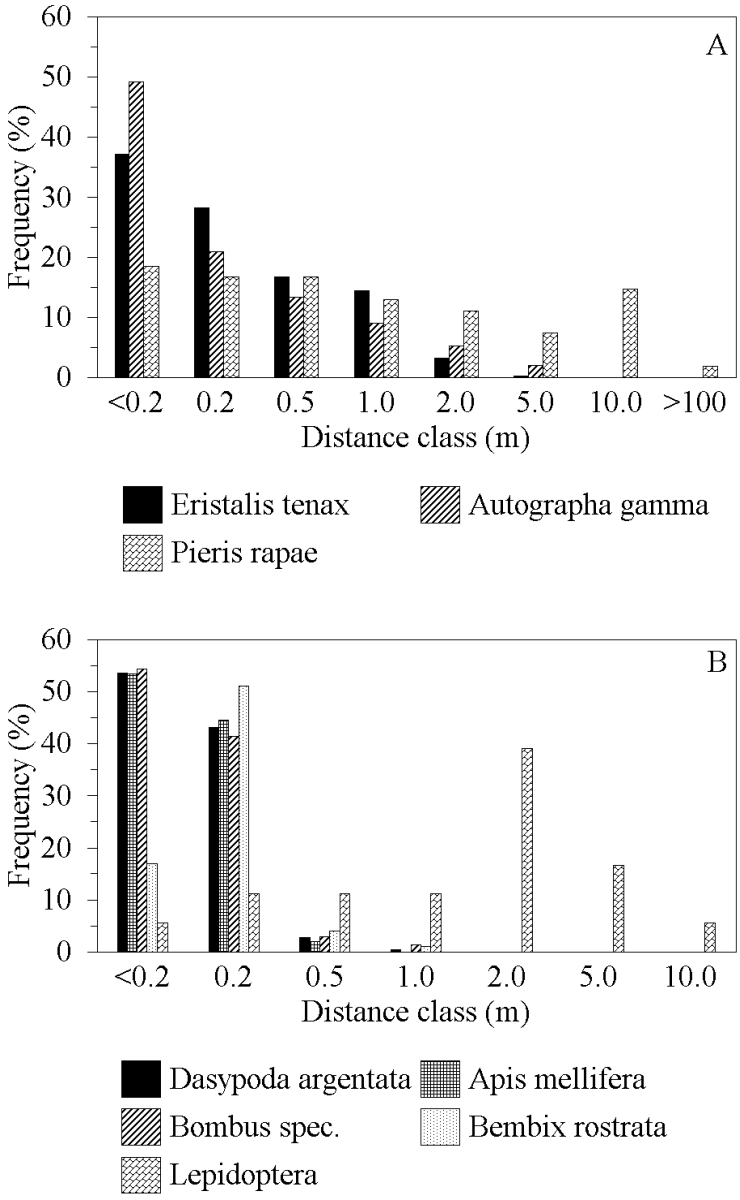

Figure 3. A. Flight distances of insects between two successive flower visits to *Scabiosa columbaria* in the Dutch population Wrakelberg on August 9, 1994. *E. tenax* n=699, *A. gamma* n=184, *P. rapae* n=182. B. In the French population Bramans cave on August 4, 1995. *D. argentata* n=220, *A. mellifera* n=200, *Bombus* spec. n=70, *Bembix rostrata* n=100, Lepidoptera n=18.

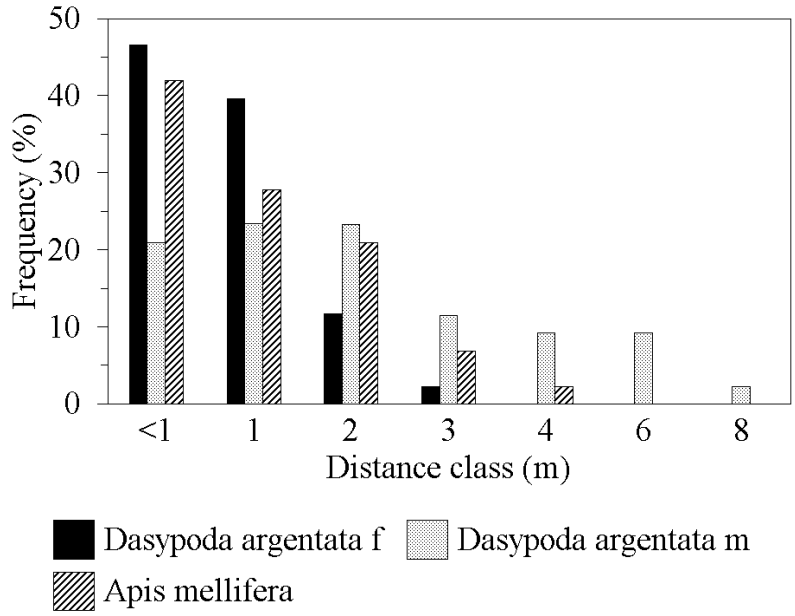

Figure 4. Overall distances (distance between the first and eleventh visited *Scabiosa columbaria* head) in the French population Bramans cave on July 30, 1997, n=43 for each species.

4. Foraging speed

Foraging speed (number of heads visited per unit time) differed strongly between insect species in the Dutch population Assen. Bumblebees visited most heads per minute; means varied between 9.6-10.8 (Table 1); this was significantly different from the other insect species (*E. tenax*, *E. arbustorum*, *H. pendulus*, *A. mellifera* and Lepidoptera) who visited 4.2-6.5 heads per minute (SNK test, $P<0.05$). The small fly species *S. geniculata* had such an extremely long residence time that foraging speed could not measured in sufficient numbers. One example of foraging speed of this small fly is 10 heads in 8'40".

Table 1 Number of *Scabiosa columbaria* heads visited per minute (mean ± s.e.) by insect species in the Dutch population Assen on two dates in 1999. Within a column only *Bombus pascuorum* differed significantly from the other species on both dates (SNK test, $P<0.05$, after ln transformation). n.a. value not available.

Insect species	August 25		September 6	
	n	heads per minute	n	heads per minute
Eristalis tenax	20	4.23 ± 0.25	6	4.33 ± 0.33
Eristalis arbustorum	16	5.66 ± 0.91	4	3.12 ± 0.28
Helophilus pendulus	15	5.72 ± 0.43	7	6.47 ± 1.66
Bombus pascuorum	14	9.61 ± 0.81	9	10.79 ± 0.92
Apis mellifera	16	5.61 ± 0.74	6	5.74 ± 1.05
Pieris rapae	12	6.36 ± 0.52	n.a.	n.a.

In the French population Bramans cave, females and males of the bee species *D. argentata*, visited most heads per minute (Table 2). Bumblebees were faster than honeybees. In summary, the following increasing sequence in foraging speed can be derived from the data (Tables 1 and 2): small flies, syrphids, Lepidoptera, honeybees, bumblebees and *D. argentata* (females and males).

Table 2 Number of *Scabiosa columbaria* heads visited per minute by insect taxa (mean ± s.e.) in two populations of *Scabiosa columbaria* in France. Between brackets the number of individuals sampled, n.a. = data not available. Per population and time of the day significant differences among species (P <0.0001, after ln transformation), are indicated by different letters, * data not included in statistical test, [1)] two bumblebee species, [2)] only butterflies.

Population	Date	Time of the day	*Dasypoda argentata* female	*Dasypoda argentata* male	*Apis mellifera*	*Bombus* spec. [1)]	*Bembix rostrata*	Lepidoptera [2)]
Bramans cave	August 4, 1995	morning	25.2 ± 1.3 (22) [a]	n.a.	7.7 ± 0.4 (20) [c]	9.5 ± 0.6 (7) [b]	7.4 ± 0.6 (10) [c]	4.7 ± 1.5 (2)*
Bramans plateau	July 28, 1997	morning	27.0 ± 1.3 (23) [a]	n.a.	5.6 ± 0.4 (30) [b]	n.a.	n.a.	n.a.
		afternoon	17.9 ± 2.6 (14) [a]	13.9 ± 10.1 (2)*	6.9 ± 0.5 (44) [b]	n.a.	n.a.	n.a.
Bramans cave	July 29, 1997	afternoon	32.0 ± 1.2 (20) [a]	12.5 ± 1.5 (24) [b]	n.a.	n.a.	n.a.	n.a.

5. Residence time

Residence time was measured for open visited heads (male and female) and for virgin female heads. For the Dutch situation, bumblebees had the shortest residence time. Means varied between 7-14 seconds for bumblebees, 13-15 seconds for honeybees, 13-24 seconds for Syrphidae, 28-41 seconds for Lepidoptera and 89 seconds for *S. geniculata* (Table 3). The foraging aim of the species (nectar or pollen) may influence residence time. Generally, Syrphidae and honeybees spent shorter periods on female heads (with only nectar) than on male heads (collection of both nectar and pollen possible), whereas Lepidoptera showed the reverse pattern (Table 4). Only for *E. arbustorum* this difference was significant ($P<0.05$, t-test after ln transformation of the data). Sample sizes for female heads were small due to the fact that the majority of heads on those days in the studied population were males (84% and 87%).

Table 3 Residence time (mean ± s.e.) of several insect species on (female and male) heads of *Scabiosa columbaria* in the Dutch population Assen on August 13 and 19 between 10.30 and 12.00 h and on September 3 between 14.00 and 16.00 h; n.a. means no data available, * data not included in statistical test, [1]) *Aglais urticae* and *Inachis io*, [2]) mainly *Aglais urticae*, [3]) *Aglais urticae*. Significant differences among insect species within one day (SNK-test after ln transformation, $P<0.05$), are indicated by different letters within a column.

Species	August 13, 1997		August 19, 1997		September 3, 1996	
	n	time in seconds	n	time in seconds	n	time in seconds
Eristalis tenax	46	20.4 ± 2.9 [b]	48	18.7 ± 2.5 [b]	38	23.8 ± 3.9 [b]
Eristalis arbustorum	46	18.6 ± 2.7 [b]	51	15.6 ± 2.3 [b]	n.a.	n.a.
Helophilus pendulus	15	12.6 ± 2.4 [bc]	46	14.7 ± 1.8 [b]	n.a.	n.a.
Bombus pascuorum	12	7.0 ± 1.6 [c]	2	13.6 ± 8.9 *	n.a.	n.a.
Apis mellifera	46	12.6 ± 1.5 [bc]	49	14.5 ± 1.5 [b]	n.a.	n.a.
Lepidoptera	46 [1)]	41.4 ± 1.5 [a]	46 [2)]	28.46 ± 3.0 [a]	11 [3)]	41.2 ± 13.9 [b]
Syrphus balteatus	n.a.	n.a.	n.a.	n.a.	41	17.8 ± 6.6 [b]
Siphona geniculata	n.a.	n.a.	n.a.	n.a.	26	88.8 ± 41.8 [a]

Table 4 Residence time in seconds (mean ± s.e.) of insect species on female and male heads of *Scabiosa columbaria* in the Dutch population Assen on August 13 and 19, 1997 between 10.30 and 12.00 h. For *Helophilus pendulus* and *Bombus* spec. no sufficient data for both sexes of heads could be collected (compare Table 3). T-test after ln transformation of the data, * = $p<0.05$.

Date	Species	Female heads		Male heads		t-test
		n	residence time	n	residence time	
August 13	*Eristalis tenax*	7	11.4 ± 2.2	39	22.0 ± 3.3	1.51 n.s.
	Eristalis arbustorum	6	7.8 ± 1.7	40	20.3 ± 3.0	2.51 *
	Lepidoptera	5	56.0 ± 24.1	41	39.6 ± 5.5	0.61 n.s.
August 19	*E. tenax*	7	12.9 ± 3.8	41	18.9 ± 2.8	0.85 n.s.
	E. arbustorum	10	17.9 ± 3.5	41	15.1 ± 1.9	1.43 n.s.
	Apis mellifera	4	8.1 ± 1.4	42	15.1 ± 1.6	1.40 n.s.
	Lepidoptera	10	31.9 ±6.1	39	27.6 ± 3.4	1.74 n.s.

The residence time of *D. argentata* females foraging for pollen was significantly shorter (2.17 ± 0.40 sec, mean ± s.e., n=30, SNK test, $P<0.05$ after ln transformation of the data) than for individuals foraging for nectar (6.89 ± 0.70 seconds, n=37) or *A. mellifera* individuals (9.27 ± 1.10 seconds, n=49, data of afternoon August 4, 1994). Residence time of females of *D. argentata* on virgin female heads (not visited during at least 15 hours thus more nectar was to be expected) during the morning was even shorter: 0.4 ± 0.2 seconds (mean ± s.e., see Table 7); this value is significantly different from the values found for *D. argentata* males and honeybees during the morning (SNK test, $P<0.05$). Visits of females of *D. argentata* to female heads during the afternoon were much longer than during the morning (28 instead of 0.4 seconds respectively), indicating that they were now also interested in nectar. Duration of visits of males, foraging for nectar or searching for mates, was similar during the morning and afternoon, but honeybees spent more time on virgin female heads in the afternoon than in the morning (30 and 11 seconds respectively, Table 7). Differences between data collected during the morning and afternoon were more pronounced than differences between species.

Long residence times make high foraging speeds impossible: Lepidoptera and *S. geniculata* are examples of this. Also honeybees spent a reasonable time per head with a moderate foraging speed. The bee *D. argentata* shows the opposite: short residence time and high foraging speed.

In summary, the following sequence in increasing residence time can be derived from Tables 3 and 7: *D. argentata* females, *Bombus* spec., *D. argentata* males, Syrphidae, *A. mellifera*, Lepidoptera and small flies.

6. Sex preference

The preference of insects for a female or male head differed between species. The small syrphid fly *Syrphus balteatus* had a strong preference for male heads, eating pollen from the anthers. In a discrimination experiment, *E. tenax* and *E. arbustorum* showed a preference for female flowers, both during the morning and afternoon (testing binomial proportion with respectively $Z=3.07$, $P<0.001$ and $Z=2.02$, $P<0.05$, data of September 3, 1999). Bumblebees, honeybees and butterflies visited female and male flowers according to their frequency.

Females of *D. argentata* visited female and male heads according to their frequency, both during the morning and afternoon. However, honeybees showed a preference for male heads during the afternoon ($Z<3.7$, $P<0.0001$) but not in the morning. This preference for male heads is also reflected in the percentage of workers with corbiculae filled with pollen of the colour of *S. columbaria*: only 6% in the morning and 39% of the workers in the afternoon carried filled corbiculae.

7. Pollen loads on insect bodies

The total number of pollen grains (all plant species) and the fraction *S. columbaria* pollen among them varied very much between insect species (Table 5). The total body load of Lepidoptera was small and ranged from 19-72 grains and the number of *S. columbaria* pollen only from 4-24 grains. The loads of syrphids (*E. tenax*) and bumblebees were comparable in size, varying between 942-1283 grains for syrphids and between 783-1764 for bumblebees. The loads of honeybees were slightly smaller: between 474-868 grains. However, the number of *S. columbaria* grains in the loads was smallest in bumblebee loads: 4-96; in syrphid loads 153-407 and in honeybee loads 260-860. Pollen of *Origanum vulgare, Daucus carota, Centaurea* spec. and Compositae were found in the loads of insects visiting Dutch *S. columbaria* populations. The load of *D. argentata* females amounted to several thousands and contained a very large number of *S. columbaria* grains.

In summary the following sequence in increasing pollen loads can be derived from Table 5: Lepidoptera, Syrphidae, *A. mellifera*, bumblebees and *D. argentata*. For the number of *S. columbaria* pollen grains per load this sequence is: Lepidoptera, bumblebees, Syrphidae, *A. mellifera* and *D. argentata*.

Table 5 Pollen loads on insects: total number of pollen grains and *Scabiosa columbaria* grains found in body loads of insects in Dutch and French populations are given (mean ± s.e.). * two species: *Melanargia galathea* and *Melitta didyma*, both butterflies.

Population	Date	Species	n	Total load	Number of *S. columbaria* grains
Wrakelberg	August 1994	*Eristalis tenax*	14	1283.1 ± 213.3	407.1 ± 37.0
		Bombus pascuorum	4	1764.5 ± 860.6	96.3 ± 85.3
		Autographa gamma	13	18.9 ± 6.9	3.7 ± 7.0
		Pieris rapae	6	71.8 ± 33.3	23.5 ± 7.4
Kruisberg	August 15, 1996	*B. pascuorum*	3	1150.3 ± 26.9	22.0 ± 21.5
		Bombus lapidarius worker	3	783.0 ± 159.0	4.0 ± 2.5
		B. lapidarius male	10	971.8 ± 126.6	51.1 ± 15.3
		A. gamma	12	34.5 ± 7.4	5.7 ± 2.2
Wijlre	August 15, 1996	*E. tenax*	8	942.0 ± 269.8	153.3 ± 175.6
		B. lapidarius male	9	1911.3 ± 301.5	180.7 ± 55.9
		A. gamma	10	30.0 ± 7.6	4.2 ± 1.3
Bramans cave	August 4, 1995	*Apis mellifera*	10	867.8 ± 170.0	859.7 ± 168.6
		Dasypoda argentata female	10	5997.2 ± 753.2	5997.2 ± 753.2
Modane	July 27, 1997	*A. mellifera*	10	473.9 ± 122.8	260.2 ± 85.3
		D. argentata	10	4308.2 ± 1255.6	3778.9 ± 1194.6
		Lepidoptera*	11	65.6	12.5 ± 3.6

8. Pollen deposition

Pollen deposition per visit (only *S. columbaria* pollen) was strongly dependent on the duration of a visit (residence time): a long visit resulted in a larger number of pollen deposited, up to a limit of 240 pollen grains per head for *E. tenax* (Figure 5). After this time an insect may have deposited its total body load and a longer residence did not result in a higher deposition. However, to reach this limit an insect must spend a rather long time per head, at least 150 seconds for *E. tenax* (Figure 5). This very long residence time was only found occasionally for virgin female heads, which were not visited during at least 15 hours. The mean residence time on virgin heads varied from 11.8 to 36.9 seconds for bumblebees on several observation days, 2.5 to 77.1 seconds for butterflies, 0.3 to 47.1 seconds for syrphids, 11.3 to 30.1 for honeybees and 0.4-28.0 for *D. argentata* (compare Tables 6, 7 and paragraph 5).

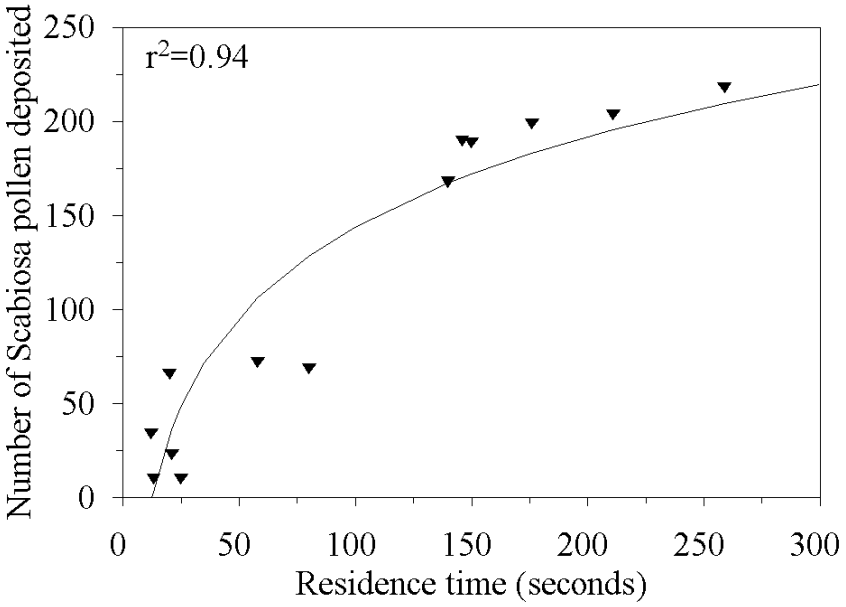

Figure 5. Relation between pollen deposition and residence time for *Eristalis tenax* foraging for nectar in a Dutch population of *Scabiosa columbaria* (Haren, October 11, 1991; line is a fitted log curve, $y=-174+69 \ln(x)$, $r^2=0.94$, $P<0.05$).

The size of the pollen load deposited varied between insect species (Tables 6 and 7). In the Dutch population, Lepidoptera deposited relatively few *S. columbaria* grains during a single visit; Syrphidae, bumblebees and honeybees deposited several tens of pollen grains (Table 6). For full seed set (one seed per flower) at least four grains are necessary (see chapter 3). In Table 6 the number of pollinated stigmas that received this minimum amount of grains are given. After a visit of *Eristalis* or a bumblebee more stigmas had this minimum amount of pollen grains than after a visit of *S. balteatus* or a butterfly. The number of stigmas with at least four *S. columbaria* grains deposited after a single visit was larger in the afternoon than during the morning for all Dutch insect species measured (Table 6).

Because pollen deposition per stigma is dependent on residence time, the deposition per stigma per second was calculated. Deposition was higher during the morning than in the afternoon for all groups of insects in the Dutch population, except for *S. balteatus*. Lepidoptera were poor pollinators concerning their total deposition and their deposition per time. Bumblebees and large syrphids were both good pollinators (Table 6). To avoid the effects of differences in residence time, also the pollen deposition after a visit of a fixed time (20 seconds) was measured in a Dutch population. Again Lepidoptera are poor pollinators compared to a syrphid species; bumblebees and several syrphid species deposited similar numbers of grains per stigma per second (Table 8).

The mean total pollen deposition per head in cages with small flies was low compared to that of *E. tenax* (15.0 ± 3.1 and 725.1 ± 202.0 respectively, mean ± s.e.). Taking into account the number of insects responsible for this deposition, the pollination value of small flies was much lower than that of *E. tenax*: each small fly deposited on average 0.7 and each *E. tenax* individual 145.0 grains per head; thus the pollen deposition of a single *E. tenax* equaled that of nearly 200 small flies.

The total number of pollen deposited per visit for *D. argentata* females during the morning was low compared to *D. argentata* males or honeybees, but due to the very short residence time, deposition per second was very high (Table 7). Also the number of fully pollinated stigmas (four or more grains) was low for *D. argentata* females. Total pollen deposition by *D. argentata* females during the afternoon was higher than during the morning; for honeybees the reverse was true. Pollen deposition per unit time for *A. mellifera* was low and for Lepidoptera very low compared with *D. argentata* but it equaled the values found in the Dutch population. Again, in general, deposition per stigma per second was higher during the morning than in the afternoon (Table 7) except for *D. argentata* males. For comparison, some values of pollen deposition per stigma per second are illustrated in Figure 6.

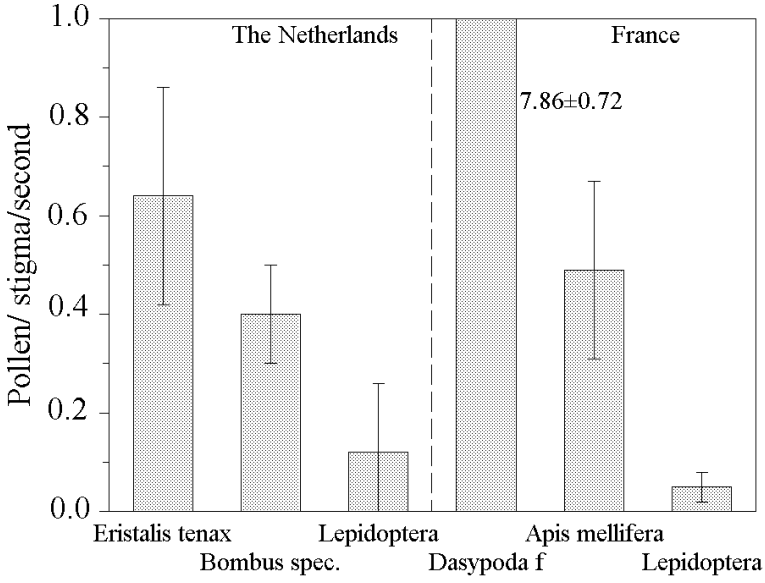

Figure 6. Pollen deposition per stigma per second on virgin heads of *Scabiosa columbaria* by various insect species in a Dutch (Assen, August 19, 1996) and a French population (Bramans cave, July 29 1997).

Table 6 Residence time (mean ± s.e. in seconds) and *Scabiosa columbaria* pollen deposition per stigma per second (mean ± s.e.) of insect species that visited virgin female *S. columbaria* heads with varying residence times in the Dutch population Assen on August 19, 1996. [A)] *Bombus pascuorum* and *B. lapidarius*, [B)] *B. terrestris*, [C)] *Eristalis arbustorum*, [D] *E. arbustorum* and *E. tenax*, [E)] *Inachis io* and *Pieris rapae*, [F)] *I. io.* Different letters within a column indicate significant differences among species; [1)] SNK test, P<0.05 after ln or (ln+1) transformation of the data, [2)] Mann-Whitney U-test.

Species	Morning					Afternoon				
	n	total number of *S. columbaria* pollen deposited per visit [1)]	number of stigmas with 4 or more pollen grains [2)]	residence time (seconds) [1)]	pollen/stigma/ second [1)]	n	total number of *S. columbaria* pollen deposited per visit [1)]	number of stigmas with 4 or more pollen grains [2)]	residence time (seconds) [1)]	pollen/stigma/second [1)]
Bombus spec.	10 [A)]	101.9 ± 27.7 [a]	8.8 ± 3.2 [a]	11.8 ± 3.0 [a]	0.40 ± 0.10 [a]	10 [B)]	169.1 ± 69.6 [a]	17.0 ± 6.6 [a]	36.9 ± 13.4 [a]	0.20 ± 0.05 [b]
Eristalis spec.	4 [C)]	25.5 ± 16.6 [b]	1.0 ± 0.8 [b]	4.4 ± 1.1 [a]	0.64 ± 0.22 [a]	6 [D)]	93.8 ± 20.3 [ab]	9.8 ± 2.6 [a]	47.1 ± 168.6 [a]	0.18 ± 0.05 [b]
Syrphus balteatus	4	10.8 ± 7.5 [b]	0.3 ± 0.3 [b]	10.44 ± 2.5 [a]	0.19 ± 0.05 [ab]	3	23.3 ± 9.2 [ac]	6.7 ± 3.7 [a]	4.4 ± 0.9 [b]	0.51 ± 0.11 [a]
Lepidoptera	10 [E)]	10.3 ± 3.4 [b]	0.1 ± 0.1 [b]	39.3 ± 16.2 [a]	0.11 ± 0.14 [b]	10 [F)]	9.4 ± 3.3 [c]	0.4 ± 0.2 [b]	77.1 ± 23.3 [a]	0.04 ± 0.01 [c]

Table 7 Pollen deposition, residence time (mean ± s.e.) on virgin female heads and number of pollen deposited per stigma per second of various insect species in the French population Bramans cave of *Scabiosa columbaria* on July 29, 1997. n = number of observations, n.a. not available; * data not included in statistical test. If insect species differ significantly during the same time of the day, this is indicated by a different letter ([1)] SNK test after ln transformation, P<0.05 or [2)] Kruskal-Wallis test).

Species	Morning					Afternoon				
	n	total number of pollen deposited per visit [1)]	number of stigmas with 4 or more pollen grains [2)]	residence time (seconds) [1)]	grains/stigma/ second [1)]	n	total number of pollen deposited per visit [1)]	number of stigmas with 4 or more pollen grains [2)]	residence time (seconds) [1)]	grains/stigma/ second [1)]
Dasypoda argentata female	14	18.4 ± 3.0 [a]	1.4 ± 0.4 [a]	0.4 ± 0.2 [b]	7.86 ± 0.72 [a]	17	50.0 ± 19.3 [a]	4.8 ± 2.1 [a]	28.0 ± 6.8 [a]	3.33 ± 0.92 [a]
D. argentata male	3	89.0 ± 61.6 [ab]	9.3 ± 7.4 [a]	17.1 ± 14.7 [a]	2.78 ± 2.67 [b]	5	72.8 ± 45.8 [a]	15.4 ± 8.1 [a]	17.2 ± 10.5 [a]	5.01 ± 1.79 [a]
Apis mellifera	4	86.5 ± 36.2 [b]	9.3 ± 4.9 [a]	11.3 ± 5.5 [a]	0.49 ± 0.18 [b]	18	54.2 ± 15.7 [a]	5.6 ± 1.9 [a]	30.1 ± 7.2 [a]	0.20 ± 0.20 [b]
Lepidoptera (butterflies)	2*	3.5 ± 0.7	0	2.50 ± 0.5	0.05 ± 0.03	n.a.	n.a.	n.a.	n.a.	n.a.

Table 8 Pollen deposition (mean ± s.e.) of insect species that were allowed to visit *Scabiosa columbaria* heads for 20 seconds in the Dutch population Assen in 1997; * observations made at 12.00 h, other observations made at 14.00 h on August 20; observations on August 29 done at 11.00 h. Differences are tested for data collected at the same time at the same day. During the morning of August 20, *Eristalis arbustorum* differed significantly from Lepidoptera in all measured characters (t-test after ln or (ln+1) transformation of the data, $P<0.005$). During the afternoon of August 20 and on August 29 insects did not differ significantly in these characters (Oneway Anova, $P>0.05$).

Species	August 20				August 29			
	n	mean of total number of pollen deposited per visit	number of stigmas with 4 or more *S. columbaria* grains	pollen/stigma/ second	n	mean of total number of pollen deposited per visit	number of stigmas with 4 or more *S. columbaria* grains	pollen/stigma/ second
Eristalis tenax	10	141.9 ± 25.1	14.6 ± 2.6	0.21 ± 0.02	13	94.2 ± 26.4	7.4 ± 2.1	0.19 ± 0.03
*E. arbustorum**	16	165.0 ± 32.3 [a]	15.0 ± 2.7 [a]	0.31 ± 0.04 [a]	10	90.3 ± 26.1	8.9 ± 2.9	0.15 ± 0.02
Helophilus pendulus	10	88.5 ± 20.7	7.8 ± 2.1	0.17 ± 0.02	15	70.1 ± 10.7	5.7 ± 1.0	0.18 ± 0.02
Eristalis intricarius	10	133.4 ± 11.2	13.0 ± 1.1	0.22 ± 0.02				
Bombus pascuorum	4	120.5 ± 32.5	11.3 ± 4.1	0.23 ± 0.04				
*Aglais urticae**	14	23.8 ± 4.0 [b]	1.3 ± 2.7 [b]	0.12 ± 0.01 [b]				

Discussion

Flowering

The species *S. columbaria* and also its close relatives *Succisa pratensis* and *Knautia arvense* have an interesting flower development. Flowers within one head show a different length (between about a half and eight days) of a neuter phase, until all flowers in a head had passed the male phase before the stigmas protruded. Flowers in all phases (male, neuter and female) contain nectar. According to Snow & Grove (1995), there exists only few species with flowers in a neuter phase in which the flowers remain open and continue to provide nectar without dispersing or receiving pollen.

Each flower produces about 340 grains per anther, so total pollen production per flower is 1360. The maximum number of *S. columbaria* pollen that a single stigma can hold is about 30 grains. Thus a maximum of 2.2% of the total number of pollen grains produced can reach conspecific stigmas. This value is well in the range of the 0.01-2.9% given by various authors (Snow and Roubik 1987; Galen and Stanton 1989; Stanton et al. 1991; Thøstesen and Olesen 1996; Rademaker et al. 1997; Larson and Barrett 1999).

Insect activity during the day and behaviour

Scabiosa columbaria is visited by a large number of insect species of various orders. The flowers of *S. columbaria* have such morphology and size that many insect species touched anthers and stigmas while foraging for pollen and/or nectar on *S. columbaria*. Most insect species were active during a larger part of the day but they were differently sensitive for weather conditions. Bumblebees were active already during clouded weather, sometimes honeybees too (see also Corbet et al. 1993; Herrera 1997) but Syrphidae, *D. argentata* and butterflies were mainly active during sunny hours. This variability in insect behaviour implies that field observations depend on variable conditions, which make absolute comparisons between populations hardly possible.

Residence time

Increased residence time may result in a larger number of pollen deposited (Thøstesen and Olesen 1996) but if residence is very long the number of deposited pollen does no longer increase, indicating an exhausting effect (in Figure 5 this point was reached after c.150 seconds). This effect was probably only important for the first visits during the day when nectar volumes were large but not later when nectar became depleted and residence times were short. In general, residence time on virgin heads in the afternoon was longer than during the morning but pollen deposition per stigma per second was lower (Tables 6 and 7). Residence time as a discriminating character between insect species with respect to the evaluation of their pollination value is of no value because a longer residence time is not accompanied with a higher deposition per second (Table 7, compare Lepidoptera with *D. argentata*). To avoid the complication of differences in residence time we allowed insects to visit a virgin female head for 20 seconds (mean residence time on open visited female heads during the morning varied for syrphids between 8 and 11 seconds and for Lepidoptera it was 56 seconds, Table 4). During these 20 seconds Lepidoptera had deposited the larger part of their *S. columbaria* pollen load but compared to the other insect species only a small number of pollen grains (compare Tables 5 and 6).

Pollen deposition and sex preference

To evaluate the pollination value of insect visitors, several components of their pollination behaviour were compared. Deposition per stigma per second seems a reasonable measure to compare insect species (Figure 6) but also this value is influenced by environmental circumstances: during high humidity, e.g. anthers remain closed. Tables 2, 6 and 7 demonstrate large differences between morning and afternoon data, collected in the same populations. At all moments during the day female heads become receptive and the ratio between female and male heads is continuously changing. For instance, during the morning we observed 17% females and during the afternoon 26.3%. During the afternoon new heads started to flower, some of the heads flowering as male became female and female heads during the morning, wilted during the afternoon. It is difficult to predict at what time of the day most flowers will be pollinated; at all moments during the day pollen grains and receptive stigmas are available. Most insect species deposited more grains per stigma per second during the morning than during the afternoon both in the Dutch and in the French populations. A preference for female heads during the afternoon shown by two frequently occurring syrphid species (*E. tenax* and *E. arbustorum*) may increase their importance in pollination during the afternoon. Females of the specialist bee *D. argentata* did not prefer male to female heads. However, the residence times on male and female heads differed. Apparently, this species can distinguish between the sex of heads only by touching them. This short touch of the female head resulted in pollination. Honeybees on *S. columbaria* showed a preferences for male heads during the afternoon, also reflected in a higher percentage of corbiculae filled with *S. columbaria*, but no preference during the morning. During the morning they mainly foraged for nectar, present in male and female heads.

Pollination value

In Table 9, four behavioural characteristics to be believed of main importance for the pollination value, both qualitatively and quantitatively, are ranked for five insect taxa, based on the data presented in this chapter. Flight distance is important as measure for the distance pollen can travel (qualitative aspect). Number of heads visited per minute gives an indication of the number of pollinations an individual of an insect species can make during the day (quantitative aspect). The purity of the body load is an indication of the qualitative value of an insect visit; a low purity will result in a high heterospecific pollen deposition. A high deposition of *S. columbaria* pollen deposited per stigma per time is a qualitative aspect of the value as pollinator. Various *S. columbaria* populations can be served by a different aspect. For instance, for small populations the input of pollen from another population may be very important and visitation by butterflies contributes to this aspect. Flight distances between visited flowers do not completely reflect the mobility of insects and their importance of inter-patch flights. Syrphids are continuously flying between flowers in a patch and between patches, resulting in a low recapture percentage of marked individuals (c. 4%). Both syrphids and butterflies do not have nest duties, allowing them to fly large distance. Bees are restricted in their foraging radius by the fact that they have to return to the nest. Foraging speed (number of heads visited) in combination with the abundance of insect species determines the frequency of visits on a population level; this aspect is described in a separate paper.

Also the frequency of occurrence of the behaviour is important. An occasionally occurring visit over a large distance may be enough for the required input of foreign genes in a small population, but the purity of a pollen deposition is important for every visit.

Table 9 Comparison of several behavioral characteristics of five important taxa of visitors of *Scabiosa columbaria*. The marks 1-5 indicate their significance for cross pollination: 1 indicates the best performance, 5 the worst (figures are derived from the morning values of Tables 5-7).

Insect taxon	flight distances	number of heads per minute	purity of pollen load	number of *S. columbaria* pollen per time deposited
Syrphidae	3-5	3-4	4	2
Bombus spec.	3-5	2	5	4
Apis mellifera	3-5	3-4	2	3
Dasypoda argentata	2	1	1	1
Lepidoptera	1	5	3	5

The specialistic bee species *D. argentata* scored high for all four components (Table 9), so we conclude that this species is a very good pollinator of *S. columbaria*. Although the number of fully pollinated stigmas per visit was low, they made many visits, which may result in a high paternal diversity. The size of the body load was never a limiting factor. Summarizing, *D. argentata* females were rapid foragers, with a high purity load with probably a large variation in origin of pollen donors. In addition, males had large flight distances, which also contributed to the variation in origin of pollen donors.

Honeybees and syrphids are comparable as pollinators, both having a moderate pollination value but they differ probably in the amount of inter-patch flights. Honeybees are rather restricted in the surface of their foraging area but syrphids are not. Several other studies concluded that individual honeybees are moderate pollinators (Kwak 1980; Sugden 1986; Herrera 1987; Westerkamp 1991; Willmer et al. 1994; Roubik 1996) but due to their large numbers they are sometimes responsible for most pollinations (Sugden 1986; Herrera 1989). Honeybees are flower constant in many cases but in the population Modane their body load contained 50% heterospecific pollen (Table 5), indicating that during their foraging trips at least two plant species were visited.

Bumblebees have a slightly higher pollinator value than syrphids if we consider the four components of Table 9. Surprising is that the body loads of bumblebees, which are considered as relatively flower constant (Heinrich 1979) are very heterogeneous with only 0.5-11% *S. columbaria* pollen, even less than found on syrphids. Part of the bumblebees were males which are probably less flower constant than workers but also workers had a very low percentage of *S. columbaria* pollen (only 0.5%, Table 5) compared to other studies (71.5-92.3% conspecific pollen in Petanidou et al. 1995a; 93% in Petanidou et al. 1995b). Pollen loads were collected in natural populations where many other plant species were also flowering. Pollen deposition measurements were done in the artificial population Assen where bumblebees were specialized on *S. columbaria* in the absence of other flowering plants. Both in natural and artificial populations, we never observed worker bumblebees with corbiculae filled with *S. columbaria* pollen, indicating that they visited *S. columbaria* only for nectar. Thus, bumblebees had the potential to be good pollinators measured in our artificial population, but in natural populations they carried so few *S. columbaria* grains that

their importance as pollinators of these natural populations was decreased. Bumblebees were active during more hours per day than other visitors, starting earlier and continuing longer, and were also active during less sunny weather. Jennersten et al. (1991) found that the major difference between bumblebee castes was their activity periods. Workers were active the entire day but males foraged predominantly later during the day; males and workers traveled similar distances in the field.

Syrphids were the most common visitors in the Dutch *S. columbaria* populations. For several aspects in relation to pollination they were more or less as good as bumblebees (see Table 9). One disadvantage of syrphid (and also bumblebee) pollination of *S. columbaria* is the large number of heterospecific pollen deposition. Its effect on seed set of *S. columbaria* is unknown but at least the foreign pollen occupied space on the stigmas. Also Olesen & Warncke (1989a) found that syrphids carried heterospecific pollen (23.7-25.2% of the total load was heterospecific) but these authors found that for butterflies (*Zygaena trifolii*, a day active moth, also observed as a visitor of *S. columbaria* in the French populations) this was 91.5%. Similarly, Kearns & Inouye (1994) found that flies deposited 23% heterospecific pollen. Little is known about the flower constancy of syrphids. Goulson & Wright (1998) found marked floral constancy for two pollen-foraging syrphid species in a mixed plant community. McGuire & Armbruster (1991) found that syrphids (species not mentioned) strongly preferred *Saxifraga reflexa* to *S. tricuspidata* and that interspecific transitions by syrphids were uncommon.

Small flies in cages deposited a very small number of pollen grains on *S. columbaria* heads compared to the deposition by *E. tenax*. Also the number of heads visited per minute was very low (Table 3) and their pollen carrying capacity was expected to be small. These features combined make this fly species an unimportant pollinator considered at an individual base.

Lepidoptera are important as long distance vectors being the only group regularly flying more than 10m between successive flower visits. However, the number of pollen they deposited was restricted compared to the other species. Schmitt (1980), Herrera (1987) and Olesen & Warncke (1989b) also found that butterflies were important as long-distance pollinators; their occasional long flights have a strong potential to increase neighbourhood size (Schmitt 1980; Olesen and Warncke 1989b). Moths and butterflies differed in size of the total load and the number of *S. columbaria* grains (Table 5). Moths did carry fewer grains but unfortunately we could not measure their pollen deposition due to their low abundance when the measurements were done.

Insect species differed in the frequency distribution of pollen grains deposited on the stigmas. After a visit of a honeybee, bumblebee, *D. argentata* male or a syrphid (*Eristalis* or *Helophilus* species) between 5.7 and 17.0 stigmas had received the minimal number of four pollen grains for the development of a single seed (Tables 6,7). After a visit of an individual of Lepidoptera, or of the syrphid *S. balteatus* or of the bee *D. argentata* females, this value was lower, between 0 and 6.7. Especially *S. balteatus* and Lepidoptera pollinated only a small number of stigmas per visit. Thus none of the insect species pollinated a head completely during a single visit, due to either short residence times and/or due to a shortage of *S. columbaria* pollen on the body of the insects (see Tables 3,4, 6 and 7 for residence times and total pollen deposition, and Table 5 for the number of *S. columbaria* pollen on bodies). To achieve full seed set, heads must be visited several times during the receptive phase, which lasts generally only a single day. For all populations investigated in this aspect, we found that heads received indeed more than one visit per day with a maximum of 95 visits during six hours of insect activity in a small population (unpubl. data).

Summarizing, this study shows that insect taxa differ very much in their pollination value of *S. columbaria*. Each taxon contributes differently to the various components of quality and quantity involved in the pollination of *S. columbaria*. This emphasizes the importance of a divers pollinator guild for complete and high quality pollination of a plant species.

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Comparison of different measures of gene flow in an experimentally fragmented population of insect-pollinated *Scabiosa columbaria*

With M.M. Kwak, F.J. Weissing and R. Bijlsma

Summary

The amount of gene flow is an important factor, affecting the level of genetic erosion after fragmentation of a formerly large population. For many animal-pollinated plants, gene flow occurs mainly by pollen, and is therefore depending on the foraging behaviour of their pollinators. Several different methods exist to estimate gene flow by pollen and their relative performance was assessed under semi-natural conditions. Pollen flow was measured between patches of *Scabiosa columbaria* by means of fluorescent dye powders, direct pollen number counts and allozyme markers. The main pollinators were bumblebees and syrphid flies. Variation between fields and days was large for all methods. High dispersal of fluorescent dye powder was not always reflected in high gene flow, as measured by allozyme analysis and vice versa. Nevertheless, when averaged over multiple replicates, dispersal patterns of fluorescent dye powder and allozyme alleles were almost identical. Estimates of pollen flow, based on dispersal of pollen grains were qualitatively similar to dispersal of dye powder and allozymes, although quantitative estimates of gene flow were somewhat higher. No significant effects on seed set were detected. The consequences for estimation of gene flow in fragmented habitats are discussed.

Introduction

Many plant species depend for their reproduction and gene dispersal on insect pollinators. Not surprisingly, plant-pollinator interactions have been the subject of investigations for several centuries (Baker 1983). During the last decades human activities have caused significant reductions in population size and increasing fragmentation of the remaining plant populations. This undoubtedly will affect plant-pollinator interactions (Stanton et al. 1991; Rathcke and Jules 1993; Conner and Neumeier 1995) and consequently change reproductive and gene flow patterns. Because such pollinator-mediated changes in reproduction and gene flow may have important consequences for the persistence of plant populations, these processes have become a topic of special interest in recent years (Jennersten 1988; Olesen and Jain 1994; Van Treuren et al. 1994).

The degree of isolation of individuals in a fragmented plant population is very important in the context of its vulnerability to stochastic processes. Increased levels of isolation between subpopulations lead to a higher risk of extinction because of demographic, environmental and genetic stochasticity (e.g. Lande 1988). If we neglect the effects of seed dispersal and concentrate on gene flow between existing populations, genetic stochasticity will be a major factor influencing population vulnerability. With decreasing population sizes and increasing isolation, genetic drift will become more important and (bi)parental inbreeding will increase. As a result, populations will suffer from enhanced inbreeding depression and probably negative fitness effects related to reduced genetic variation. The combined negative effects of changes in the genetic composition of small, isolated populations are called genetic erosion (Van Treuren et al. 1991; Van Treuren et al. 1993a). The impact of genetic erosion is highly dependent on the effective population size, and thus on the degree of isolation between (small) subpopulations.

Several methods have been developed to estimate isolation in natural populations of flowering plants, varying from the analysis of the genetic structure of the plant population to direct tracking of dispersal of genes, pollen or pollinators. However, these different techniques for measuring gene flow have been compared only occasionally, complicating the evaluation of their relative reliability for estimating effective gene flow.

Many studies have used measures of genetic differentiation between plant populations to estimate the amount of genetic exchange between them (e.g. Van Dijk 1987; Campbell and Dooley 1992; Rasmussen and Brødsgaard 1992; Westerbergh and Saura 1994; Latta et al. 1998). Estimates of gene flow based on the genetic differentiation between populations, are much larger than those obtained from direct observations. However, results of these studies on genetic differentiation might be confounded by other processes than gene flow alone. For example, founder effects or selection will also influence the amount of genetic differentiation between populations (Slatkin 1987; Campbell and Dooley 1992). Direct observation of the exchange of genetic marker alleles is less hampered by these limitations. Handel (1982,1983), using a morphological marker, found that pollen flow is very restricted, even within a single population and may highly depend on local conditions. Movement of genetic markers between populations has been observed to occur at somewhat larger spatial scales (measured in meters, not in the number of conspecific individuals), but was found to be still restricted to approximately one kilometer, depending on the plant species under study (Ellstrand and Marshall 1985; Ellstrand et al. 1989; Skogsmyr 1994). The development of molecular techniques, such as DNA fingerprinting and microsatellite analysis, has increased the number of available genetic markers, but such studies are still expensive and time-consuming and thus limited in sample size. Moreover, these methods may easily miss the highly incidental and unpredictable gene flow over long distances, which has important consequences for overall patterns of genetic variation (see Slatkin, 1985 for review; Neigel, 1997).

Alternative estimates of gene flow can be obtained by tracking dispersal of pollen or pollen analogues or by tracking flight patterns of pollinating insects. For large pollinators with a limited foraging area, like (bumble)bees and hummingbirds, flight distances between sequential flower visits are relatively easy to obtain but they generally underestimate gene flow distances compared to genetic markers (Schaal 1980; Ennos and Clegg 1982; Thomson and Thomson 1989; Karron et al. 1995b). Carry-over of pollen might be an important cause of this discrepancy. Due to carry-over, pollen is transported to several subsequently visited flowers instead of only to the first one, resulting in an increase in pollen dispersal distance compared to the flight distance between subsequent flower visits. Still, pollen dispersal is often restricted to distances of only a few tens of meters (Thomson and Thomson 1989; Pleasants 1991; Nilsson et al. 1992).

In the absence of specific pollen markers, direct observation of pollen flow is impossible. In these cases, one often has to rely on indirect estimates using for example fluorescent dye powders. Dispersal characteristics of pollen and pollen analogues have been compared in carry-over experiments. In most studies a qualitative agreement in dispersal behaviour between fluorescent dye powder and pollen has been found, both declining in a similar way with distance or the sequentially visited flowers (Campbell 1985; Thomson et al. 1986; Waser 1988; Fenster et al. 1996; Rademaker et al. 1997). However, the absolute amounts which are dispersed might be different for pollen and dye powders, probably in relation to differences in adherence to pollinator and stigma and in the initial amount available in the donor flower (Snow et al. 1996).

To compare relative pollen dispersal under different conditions this variation in absolute amounts dispersed is not important and the use of fluorescent dye powders as pollen analogues is generally justified.

Few studies have compared dispersal of pollen (or pollen analogues) with effective gene flow. Post-pollination events like pollen competition, selective abortion or inbreeding depression may modify the success of pollen from different source plants, thereby changing the dispersal distance of successful pollen. In two studies dispersal of fluorescent dye powder and genetic markers were compared. In an experimental setup using transgenic *Brassica*, Cresswell et al. (1995) measured flight distances of bumblebees and honeybees. These results were combined with data from a carry-over experiment with fluorescent dye powder to predict the pollen dispersal distance. Direct measures of gene flow by genetic marker alleles from the transgenic *Brassica* plants were found to be more restricted than those estimated by flight distances and pollen carry-over (Cresswell et al. 1995). In contrast to these results, Campbell (1991) found that dye powders underestimated gene flow by genetic marker alleles. She used paternity analysis within three natural populations of *Ipomopsis aggregata* to estimate gene flow distances by hummingbirds and compared these estimates to dispersal estimates obtained by fluorescent dye powder (Campbell 1991). The surprisingly high gene flow, compared to the estimates using fluorescent dye powder, was probably due to occasionally long distance flights by infrequently visiting hummingbirds.

For all estimation methods it is generally found that gene flow by pollen in insect-pollinated plants is restricted, but quantitative estimates may differ between estimation methods. Thus far, most comparative studies have been done in single (large) populations or in carry-over experiments. However, to assess population vulnerability after habitat fragmentation, the quantification of gene flow in the context of fragmented populations is crucial. Despite its importance in conservation biology, the reliability of different techniques to estimate gene flow in fragmented populations is largely unknown. In this study, we compare several estimates of gene flow in a fragmented population, under semi-natural conditions. Estimates of dispersal of pollen and pollen analogues (fluorescent dye powder) and of effective gene flow by allozyme markers between small and isolated patches of *S. columbaria* are made simultaneously. We then systematically investigate the differences between these estimates of pollen dispersal and gene flow, and evaluate the use of the various methods for different purposes.

Material and methods

Plant species and experimental setup

Scabiosa columbaria (Dipsacaceae) is a gynodioecious, protandrous perennial of dry calcareous grasslands, that is currently endangered in The Netherlands (Van Treuren et al. 1991). Its flowers are arranged in heads of 40-100 hermaphroditic flowers, each containing one ovule. Heads start flowering in male phase for a few days, during which new flowers open continuously. When all flowers have opened, the whole head enters the female phase. Usually heads are female for only a single day, but this can be extended to two days when the head remains unpollinated. *Scabiosa columbaria* is dependent on insect pollinators for fertilization of the ovules. Although the plant is self-compatible, outcrossing rates in natural populations are very high and artificial selfing severely reduces the percentage developed seeds (Van Treuren et al. 1993a).

Experimentally, we constructed two populations each with three small patches of potted plants of *S. columbaria*. The patches consisted of 30 heads each, were linearly arranged and separated by 25m distance within the two fields (Figure 1). From previous experience we know that this distance is sufficiently large to restrict the amount of gene flow between the patches considerably, compared to within-patch gene flow. In the first field (patches 1-3), each patch was marked by a unique allozyme genotype and a different colour of fluorescent dye powder, to allow comparison of dispersal of genes and pollen analogues within and between patches. In the second field (patches 4-6), all male phase heads in the outer patches (4 and 6) were emasculated every 30 min. to prevent within-patch pollination and only the central patch (5) functioned as a source of pollen and dye powder. In this case, dispersal of pollen grains could be directly compared with estimates based on pollen analogues and genes. Comparison between both fields allows estimation of the importance of within-patch pollen flow relative to between-patch pollen flow. For these comparisons transport of pollen (analogues) and genes to the outer patches within both fields is expressed relative to the amounts in their central patch (2 or 5 respectively).

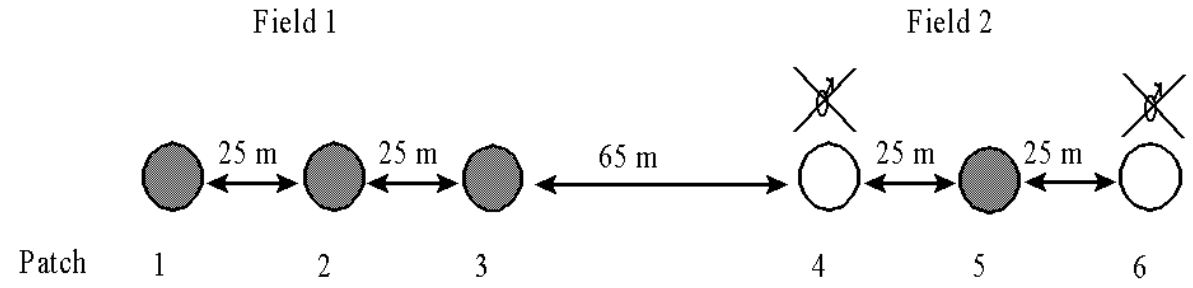

Figure 1 Experimental setup, consisting of two fields with each three patches of *Scabiosa columbaria*. Filled circles indicate patches with 20 ♂ and 10 ♀ heads. Each patch had a unique colour of fluorescent dye powder. Female flowering plants were all homozygous for the common allozyme allele, male flowering plants were heterozygous for the common allele and a unique allele. Open circles indicate patches with 20 emasculated ♂ and 10 ♀ heads, these plants were all homozygous for the common allele. Populations are located on 2 different fields, separated by a ditch.

The two replicated populations were located on different fields, both without other flowering plant species. The distance between the two closest patches of the two fields was limited to 65 m (Figure 1). All patches had an equal size, consisting of 20 male phase heads and 10 female phase heads, which were virgin at the start of the experiment. The experiment was repeated 5 times in August and September 1996. Although no selection for weather conditions was made, weather variation between days was small (Table 1). Each replicate observation started between 10 and 11 AM, while after 5 to 6 hours most stigmas contained pollen and the experiment was terminated. The female phase heads were bagged to prevent further pollination. After two weeks seeds were collected and the numbers of developed and failed seeds were counted.

Table 1 Details of the experiment and weather conditions for each day of observation. Observation rounds were made in the morning and in the afternoon. Orientation of the experiment: line from patch 1 to 6 goes from WestNorthWest to EastSouthEast.

Day (1996)	Sunshine	Max. temperature (°C)	Wind speed (m/s) and direction	Duration	Number of rounds (a.m., p.m.)
August 16	0 h	19.7	2	3 h	30, 15
August 22	5.5 h	24.5	2	6 h	30, 30
September 2	9.3 h	20.3	2	5 h	30, 30
September 6	6.1 h	19.5	3 (N)	6.5 h	30, 30
September 11	2.4 h	15.8	4 (W)	6 h	30, 30

Insect visitation

In the morning and in the afternoon we made 30 point observations of insect visitation for all six patches (60 observation rounds in total). Insects were classified into 6 groups, given in order of pollination efficiency (see chapter 2): (1) Apidae (almost exclusively bumblebees), (2) large syrphid flies (Diptera, *Eristalis* and *Helophilus* species), (3) other flies (Diptera, including *Rhingia campestris*), (4) *(Epi)syrphus* species (Diptera), (5) Lepidoptera and (6) small *Siphona* flies (Diptera). Except for *(Epi)syrphus* species all visitors are known to collect nectar, and did indeed not react to emasculation treatments (not shown). Previous experiments have shown that bumblebees and large syrphid flies are efficient pollinators of *S. columbaria*, whereas the other visitors contribute very little to its pollination (Kwak and Velterop 1997). To get insight into flight patterns and pollen transport we marked individual bumblebees. Marking of syrphid flies was highly ineffective, due to the constant turnover of individuals (Ottenheim, pers. comm., and own observations).

Dispersal of pollen and fluorescent dye powder

Using a magnifying glass (20x), the number of pollen grains (size 50-70 µm, pers. comm. G. Romeijn) on all stigmas was determined at the end of each day. Given the high pollen availability in non-emasculated patches, the number of pollen grains per stigma was roughly estimated for these patches, while we used direct counting of pollen grains on all stigmas for emasculated patches. Differences in stigmatic pollen load between patches were tested non-parametrically (Kruskal-Wallis over 6 patches, tested per day).

At the start of the experiment, fluorescent dye powder (Radiant Color NV, Houthalen, Belgium) was applied as a pollen analogue to five male phase heads in all non-emasculated patches (1,2,3,5). Each patch received dye powder of a different colour and colours were rotated between the five experimental days. At the end of the day, 10 stigmas per female phase head were sampled in each patch, mounted in glycerin on a microscope slide and stored for later analysis. For all patches, the number of fluorescent dye particles per stigma was determined for each colour, using a fluorescence microscope (400x). Because the number of dye particles is expected to decay exponentially along a visitation sequence (e.g. Campbell 1985), we used a logarithmic scale. Small numbers of dye powder were directly counted (up to 16), while larger amounts were estimated in classes of exponent 2 (<32, <64, <128, ..., <2048 particles per stigma). Based on these 100 stigmas per patch, the mean number of dye particles per stigma was estimated for each patch, using log-transformation. Because the applied amount of dye powder

will vary between heads, patches and days, the dispersal of dye powder to other patches is presented relative to the amount deposited in the source patch.

Seed set and gene flow

After about two weeks the seeds were collected and the percentage of developed seeds was determined (Van Treuren et al. 1994). To assess gene flow directly we used allozyme analysis of all developed seeds. Because we had insufficient numbers of homozygous rare genotypes, we had to use male phase plants, which were heterozygous for a common allele (*N*) and a patch-specific allele (*S*, *I*, *M* and *F* respectively) at the *Glucose-phosphate-isomerase* locus. All female phase plants were homozygous for the common allele. Each source patch had a different patch-specific allele, which was rotated over the five experimental days. For more information about electrophoretic techniques and allele distinction, see Van Treuren and Bijlsma (1992). The paternal patch could be determined for those seeds, which had received a patch-specific allele (about half of the seeds, since heterozygous males were used). Gene flow to other patches is expressed relative to the amount of gene flow within the source patch.

Results

Insect visitation

Species composition of visiting insects was relatively similar in all patches (Figure 2A). Most syrphid species visited *S. columbaria* for nectar and did not discriminate between emasculated and intact male phase heads. Bumblebees foraged mainly for nectar, but they visited patches with intact male flowers (1,2,3,5) slightly more often than patches with emasculated flowers (4,6) (binomial proportion, $P<0.05$). This might have reduced pollen flow in field 2, especially in August when most bumblebees were present. Only *(Epi)syrphus* species actively consumed pollen of *S. columbaria* and these species preferentially visited patches with intact flowers over patches with emasculated flowers ($P<0.0001$). Butterflies were frequently searching for mates instead of foraging for food and they also visited patches with intact flowers more often ($P<0.0001$). *Siphona* had a slight preference for patches with emasculated flowers ($P<0.05$), possibly because these very small flies are disturbed by the anthers of non-emasculated male flowers.

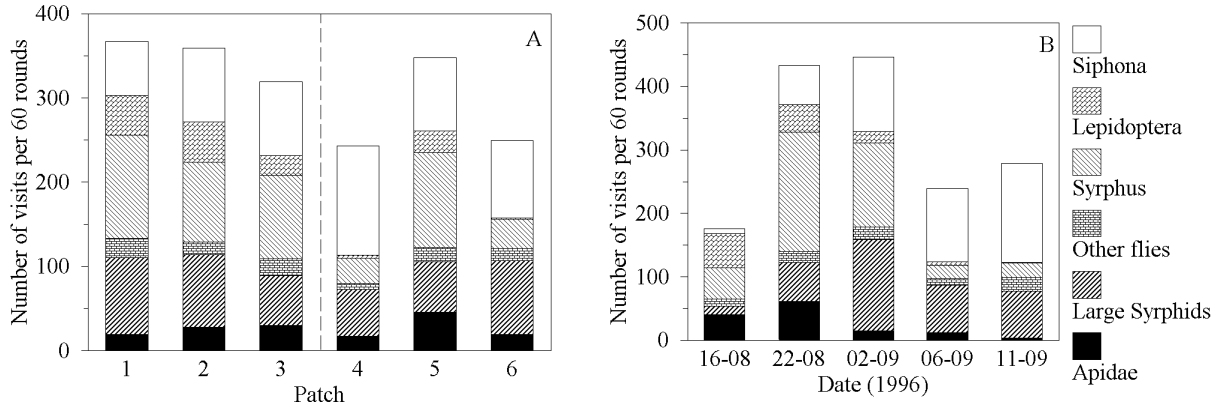

Figure 2 Number of visits per insect group during observations in 60 rounds. (A) Total number of observations per patch, averaged over five days in 1996. Patch 4 and 6 had been emasculated. (B) Total number of observations per day, averaged over 6 patches.

Although all replicates were performed within a month the species composition clearly changed over the season ($\chi^2 = 3592$, $P<0.001$, Figure 2B). Of the important pollinators of *S. columbaria*, bumblebees declined in numbers and were replaced by large syrphids (*Eristalis* species), retaining pollination of *S. columbaria* high. Unfortunately, marking of bumblebees was restricted due to practical limitations and consequently observations of individual pollinator flights were highly incidental. On August 22 the largest number of bumblebee individuals was observed, with the highest number of flights between patches, but almost exclusively within fields (Table 2). On September 2 more flights between the two fields were recorded, made by only a single bumblebee. This observation illustrates the large number of flights, which can be made by only a few bumblebees, and the importance of individual marking of flower visitors. Later in September no bumblebee flights between fields were observed. Since only a small fraction of all visitors could be marked, the potential gene flow between patches is high, especially for the first experiments. Some gene flow between the two fields has to be expected.

Table 2 Movements of individually marked bumblebees. Two sequential observations of a marked bumblebee in different patches are defined as a movement. Between brackets the number of bumblebee individuals contributing to the movements within each category is given.

Day (1996)	Number of observed individual bumblebees	Movements		
		Within field 1	Within field 2	Between fields
August 16	2	12 (2)	0	0
August 22	11	68 (6)	29 (5)	1 (1)
September 2	3	8 (3)	5 (1)	12 (1)
September 6	1	0	4 (1)	0
September 11	0	-	-	-

Pollination and seed set

Pollination intensity, measured as pollen deposition per stigma, was high but variable on all days (Figure 3). Patches with non-emasculated flowers (field 1 and patch 5) generally received between 5 and 10 pollen grains per stigma. Pollen deposition was significantly lower in patches with emasculated male flowers (4,6), which depended on between-patch pollen transport for pollination (Kruskal-Wallis over all patches, all days combined $P<0.0001$). Clearly within-patch pollination occurred frequently in *S. columbaria*. There was considerable variation in seed set, but no consistent differences between experimental treatments were found (Figure 4). Variation in seed set between individual heads within a patch and between patches, fields and experimental days, was very high. Consequently, no significant differences in seed set were detected (Kruskal-Wallis).

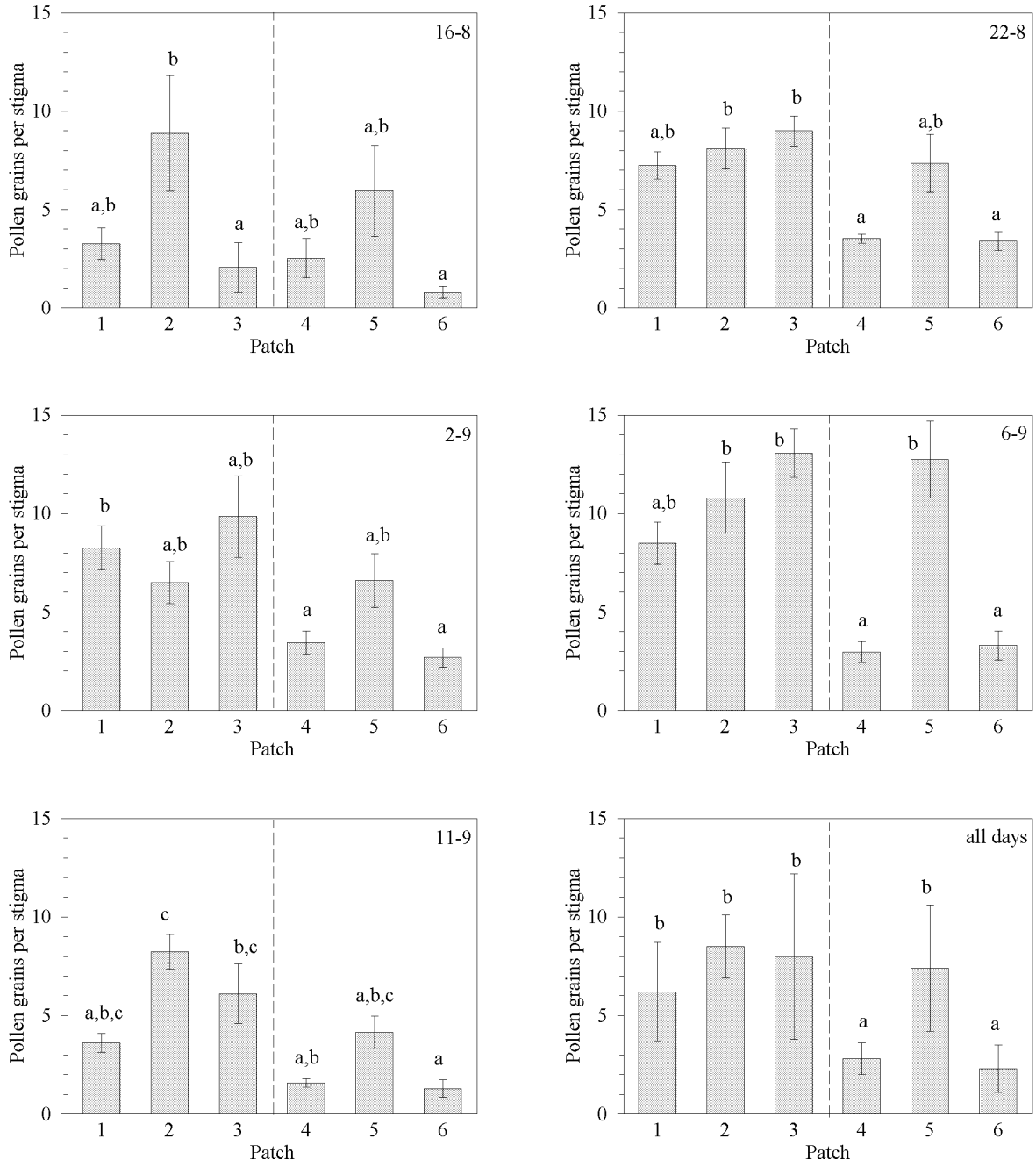

Figure 3 Mean number (mean ± s.e.) of pollen grains per stigma per patch. The upper right corners show the dates of the experiments (1996). Patch 4 and 6 were emasculated and pollen grains were directly counted at all stigmas. For the other patches estimates of the number of pollen grains per stigma were made for each female phase head. Identical letters above bars indicate means, which are not significantly different at the 5% level (Kruskal-Wallis, all patches together).

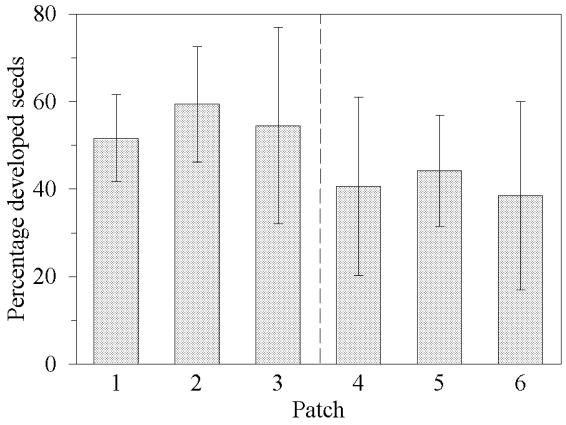

Figure 4 Percentage of developed seeds per head per patch (mean ± s.e. over the five days). Patch 4 and 6 were emasculated. In general, ten heads per patch per day were analysed.

Dispersal of pollen (analogues) and genes

Dispersal of pollen grains could only be observed directly in field 2, where the outer patches had been emasculated. Although, prevention of within-patch pollination did reduce pollen deposition on the stigmas to about one third, considerable amounts of pollen were transported over 25m (Figure 3, compare patches 4 and 6 with patch 5, Table 3). Dispersal of fluorescent dye powder to neighbouring patches within each field, varied between days, ranging from approximately 5% to 30%, compared to the deposition within the source patch (Table 3). Most dye powder was deposited within the source patch (which is set to 100%), but movement among patches within a field (over up to 50m) occurred regularly. In general, the two fields differed hardly with respect to the level of dispersal within each field, indicating that emasculation did not influence transport of fluorescent dye powder (see Table 3). No significant differences in dispersal could be detected, due to the high variation between days. Only on September 11, dye powder dispersal in field 1 seems to be lower than in field 2, but sample sizes were too small to allow statistical testing. Dispersal of dye powder between the two fields was low. The amount of dye powder, which is deposited in patches in the other field, was always less than 2.5% of the amount found in the source patch. Thus, isolation between fields was reasonably high.

Table 3 Relative dispersal of dye powder, alleles and pollen grains per receptor patch per observation day. The number of particles, found in each receptor patch, is expressed as percentage of the number deposited in the source patch. Note that field 1 consisted of three patches, which all functioned both as source and receptor. Field 2 had only a single patch functioning as both source and receptor and additionally two emasculated patches, which functioned as receptor only. Fluorescent dye powder was determined on a sample of ten stigmas for each of the ten female phase flower heads per patch. Paternity could be determined for 351, 404, 229, 303 and 72 seeds on August 16 until September 11, respectively. Dispersal of pollen grains could be measured in field 2 only. The number of pollen grains was counted directly on all stigmas in patch 4 and 6 and estimated in patch 5. n.a. = not applicable, can be scored only for emasculated patches, ** dispersal of dye powder could be measured only from source patches 2 and 3, because two indistinguishable dye colours were used by accident in patch 1 and 5.

Day (1996)		Percentage of source patch		
		Dye powder	Alleles	Pollen
August 16 **	Within field 1	15.7	14.6	n.a.
	Within field 2	**	26.1	27.5
	Between fields	0.8	1.9	n.a.
August 22	Within field 1	24.4	17.3	n.a.
	Within field 2	26.3	66.7	46.6
	Between fields	1.5	6.7	n.a.
September 2	Within field 1	4.9	4.4	n.a.
	Within field 2	8.0	41.7	47.0
	Between fields	1.4	1.2	n.a.
September 6	Within field 1	23.9	6.0	n.a.
	Within field 2	29.3	3.7	24.6
	Between fields	1.5	0.2	n.a.
September 11	Within field 1	4.4	7.2	n.a.
	Within field 2	23.4	50.0	34.5
	Between fields	2.3	2.1	n.a.

The relatively strong isolation between the two fields was confirmed by the allozyme analysis. Out of 3963 genotyped seeds, 1359 seeds were sired by a patch-specific allele, for which paternity could be assigned with certainty. Gene flow was measured for each source patch separately, by analysing the distribution of ovules sired by the patch-specific allele of that source patch. To calculate the amount of gene flow, the number of ovules sired in each receptor patch was divided by the number of ovules sired within the source patch itself. The average level of gene flow for all source patches is given in Table 3. Large variation in gene flow between different source patches, fields and days was observed. Gene flow within fields was always higher than gene flow between fields. Except for August 22, fertilization by plants from the other field was below 2.5% of the fertilizations within the source patch. The relatively high dispersal of allozyme markers between fields for August 22 was not found for dispersal of fluorescent dye powders (Table 3).

Gene flow within a field was found to be influenced by emasculation. Within field 2 dispersal of alleles was much higher than within field 1, except for September 6 (Table 3). The difference was not statistically significant, due to low sample sizes and large variation between source patches and days. Nevertheless, the absence of ‘local’ pollen within emasculated patches (4,6) increased the detection of fertilizations by pollen from the neighbouring patch (5). In patches with intact, non-emasculated flowers (field 1), the majority of the seeds (75-90%) was fertilized by pollen donors within the own patch (Table 3). Variation in gene flow between days was higher within field 2 than within field 1. The absence of ‘local’ pollen in the outer patches of

field 2, made gene flow more susceptible to stochastic variation compared to field 1. The transport of fluorescent dye powder was not affected by emasculation and dispersed relatively similar in both fields. Within field 1, dispersal of fluorescent dye powder and allozyme alleles had the same order of magnitude (see Table 3).

Averaged over five days dispersal patterns of pollen grains, fluorescent dye powder and allozyme alleles were qualitatively similar (Figure 5). High dispersal of dye powder corresponded generally with high pollen and gene flow. However, comparison of the values observed for patches 4 and 6 with those of patches 1 and 3, reveals that emasculation resulted in consistently higher estimates of dispersal levels, based on pollen counts and allozyme markers, than based on fluorescent dye powders (Figure 5, bottom). This indicates that the estimation methods were differently affected by emasculation.

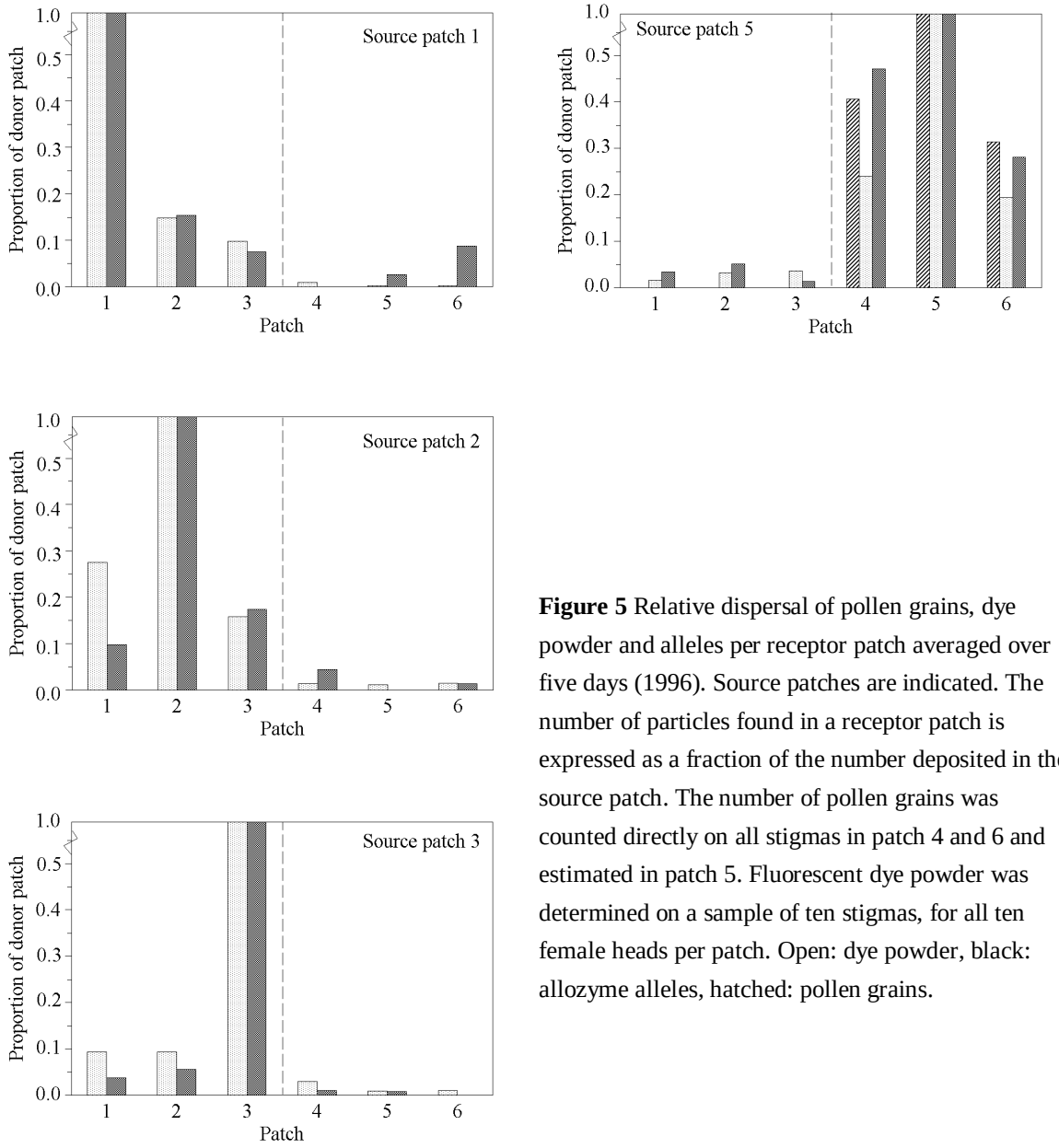

Figure 5 Relative dispersal of pollen grains, dye powder and alleles per receptor patch averaged over five days (1996). Source patches are indicated. The number of particles found in a receptor patch is expressed as a fraction of the number deposited in the source patch. The number of pollen grains was counted directly on all stigmas in patch 4 and 6 and estimated in patch 5. Fluorescent dye powder was determined on a sample of ten stigmas, for all ten female heads per patch. Open: dye powder, black: allozyme alleles, hatched: pollen grains.

Discussion

Variation between days in estimated pollen flow was high for all methods, which is in agreement with other studies (e.g. Thomson and Plowright 1980; Campbell and Waser 1989; Harder and Barrett 1996; Snow et al. 1996; Kwak 1997). Furthermore variation was partly independent for the different methods, e.g. the high level of effective gene flow between fields on August 22 was not correlated by dispersal of dye powders and a high level of dye powder transport on September 6 was not reflected in dispersal of allozyme markers. This might be related to the behaviour of the insects. When a single individual pollinator is largely responsible for gene flow, high stochasticity is introduced, depending on the individual foraging pattern. A large impact of only a single individual bumblebee was found for *Phyteuma nigrum* at the end of its flowering season (Kwak et al. 1998), and is also suggested by the bumblebee movements between our fields (Table 2). Unfortunately, syrphid flies are not useful for capture-mark-recapture studies (own data, Ottenheim pers. comm.), making it impossible to obtain more reliable data on individual foraging behaviour of these important pollinators. Especially on days with relatively low visitation rates, the insects could miss by chance the heads with dye powder, which was applied on only 25% of all male phase heads, leading to variation in dye powder dispersal. Since all male heads possessed the same genetic marker, these could not be missed by the insects. However, since the number of genotyped seeds is often limited, gene flow estimates are subjected to even higher stochastic variation than dye powders.

Each method for estimating gene flow has both advantages and limitations; therefore, the choice should depend on the specific research questions in combination with characteristics of flowers and pollinators. Tracking individual pollinators strongly depends on the presence of the right pollinators (e.g. bees, bumblebees or butterflies). Even then, additional experiments are needed to obtain insight in their relative importance for pollination, depending on characters like body load and deposition of pollen, foraging speed, flower constancy and flight distance (chapter 2; Kwak, 1997 #455). Nevertheless, mark-recapture experiments of pollinators may provide useful information about the potential for pollen flow between (sub)populations on relatively large geographic scales. More direct measures of gene flow, like dispersal of dye powders or allozyme analysis, would require enormous sample sizes, due to dilution effects with increasing distances. In cases, where the potential target area for gene flow is limited, genetic markers will give the most reliable estimates of effective gene flow and provide insight into the consequences for the genetic population structure, since they include post-pollination selection of genotypes (Snow et al. 1996). Dispersal of dye powder does not require the availability of genetic markers, but it ignores post-pollination selection of genotypes. Dye powder is an artificial pollen analogue with potentially different dispersal characteristics, which further limit its value for absolute estimates of effective gene flow. Since qualitative dispersal patterns of allozyme alleles and dye powders were similar, dye powder seems to be valuable for comparing the relative importance of gene flow under different experimental and environmental conditions. Given the highly stochastic nature of pollen deposition, however, all methods necessitate a sufficient level of replication to obtain reliable estimates of gene flow.

Habitat fragmentation may reduce the availability of pollen for fertilization of ovules. However, reduction of pollen supply by emasculation did not influence seed set in our experiment, since *S. columbaria* is visited by many different insects (bumblebees, flies, butterflies) and received on average 30 visits per head during an experimental day (including c.10 visits by important pollinators). Despite seasonal shifts in insect species composition, the high visitation resulted in a high rate of pollination of all virgin female heads, because the number of visits by bumblebees and large syrphids remained high (above 6 visits per head). The large syrphid flies are generally the most important pollinators (chapter 2; Kwak and Velterop, 1997) and they did not discriminate between patches with intact or emasculated male flowers. Even in emasculated patches the pollen load of the stigmas was usually about 2 - 3 pollen grains per stigma, which is sufficient for seed set. Figure 6 shows that initially seed set increased linearly with the number of pollen grains per stigma ($y=18.3+7,8x$, $r^2=0.32$ overall). Saturation of seed set is reached for approximately 4 pollen grains per stigma, although each flower contains only one ovule. With higher pollen loads per stigma, seed set did not increase any further. A similar relation between seed production and pollination intensity is reported for other plant species (e.g. Waser and Fugate 1986; Snow and Grove 1995). The saturation of seed set with 4 pollen grains per stigma implies that almost all stigmas received sufficient pollination for maximal seed set, even in emasculated patches. Differences in pollination intensity will not be translated in differences in seed set under such saturated conditions. Additionally, variation in seed set was very high. Part of this variation can possibly be explained by damage during handling of the heads before the seeds ripened, leading to failure of seed set. Discrimination between developed and failed seeds appeared to be difficult for some maternal plants. These methodological aspects may have contributed to the finding that no significant differences in seed set were observed.

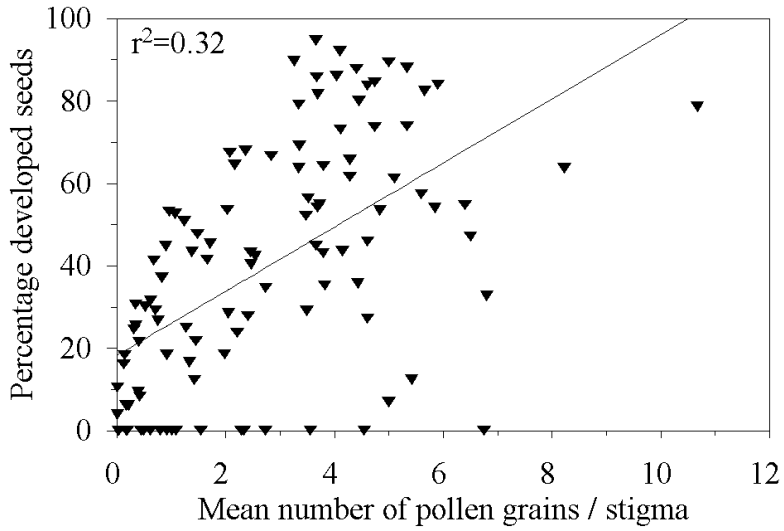

Figure 6 Percentage developed seeds per head in relation to the mean number of pollen grains per stigma. Only heads are shown for which pollen on all stigmas had been counted directly (n=108). Data for the five days are combined. Linear regression curve: $y=18.3+7.8x$, $r^2=0.32$.

We compared different methods to estimate gene flow. Dispersal of pollen is often qualitatively similar to dye powders in carry-over experiments (e.g. Campbell 1985; Waser 1988; Rademaker et al. 1997). However, conditions in our study system are quite different from those carry-over experiments, which considered mainly bumblebees. *Scabiosa columbaria* is visited by a diverse assemblage of insects, each species having its own (unknown) carry-over characteristics, that might have affected pollen and dye powder differently. Differences between dispersal of pollen and dye powder may also be brought about by size differences. Pollen grains of *S. columbaria* (50-70 µm) are much larger than dye powder particles (<5 µm), resulting in lower absolute numbers and quicker saturation of the stigmatic surface for pollen. Emasculation lowered the total amount of pollen in the system, which limited saturation of stigmatic surfaces and enhanced estimates of pollen flow, based on counting pollen grains. For dye powder particles, emasculation did not influence deposition opportunities to stigmas and the resulting estimates of pollen flow were not affected by emasculation. Such quantitative differences between methods for estimating pollen flow (as also reported by Snow et al., 1996) might hamper studies aimed at the estimation of absolute amounts of gene flow by pollen in plant populations. Nevertheless, the qualitative agreement between dispersal of pollen grains and fluorescent dye powder was reasonably high, making both methods useful for comparative studies to changes in the relative importance of pollen flow.

We found high stigmatic pollen loads in all patches. The availability of multiple pollen grains might allow competition between those pollen grains for fertilization and selection by the mother plant for pollen with the highest quality, leading to increased fitness of the seeds (Eckert and Barrett 1994). Such pollen competition can change the relative fertilization success of pollen grains from different source patches, resulting in a difference between estimates of gene flow based on the allozyme analysis on the one hand, and dispersal of dye powder particles, which do not compete with each other, on the other hand. When we prevented within-patch pollination by emasculation, the success in fertilization of between-patch pollen was increased, indicating that competition between local and imported pollen occurred. Local pollen will on average reach stigmas earlier and in larger numbers, reducing the success of pollen arriving thereafter from other patches. In natural populations of *S. columbaria* this competition with local pollen will be even larger, because natural populations consist of about 85% male phase flowers (based on the duration of male and female phase flowering), while in our experiments this was only 67%. Due to the lower availability of local pollen in our experiments, we have slightly overestimated the absolute amount of effective gene flow between patches compared to natural populations, even in field 1, where male flowers were not emasculated.

Fragmentation had no significant effects on seed set in our experiments, probably due to the high visitation rates, which resulted in high pollination intensity. We used small patches of *S. columbaria*, but with very high flower densities, making those patches attractive to pollinators. Fragmentation will often result in both smaller population sizes and reduced flower density. Reductions in density are regularly accompanied by low pollination intensity and a decline in seed set (e.g. Van Treuren et al. 1994; Kunin 1997; Kwak et al. 1998). Increased distances between patches, due to fragmentation, showed a clear increase in intra-patch fertilization success, as is also reported for *Silene alba* by Richards et al. (1999). As a consequence of the leptokurtic distribution of dispersal distances, an isolation distance of 65m (between fields) was sufficient to limit strongly gene flow between patches. A possible long-term consequence of such reduced pollen flow is an increase in biparental inbreeding in small and isolated patches, resulting in inbreeding depression and a decline in fitness. In conclusion, fragmentation affects pollinator behaviour, leading to an increase in intra-patch movements at the cost of inter-patch movement. These changes in pollinator flight patterns imply an increased biparental inbreeding and geitonogamous selfing, which may have negative fitness effects (Van Treuren et al. 1993a).

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Pollen and gene flow in insect-pollinated populations of *Scabiosa columbaria*: the effects of distance and barriers

With M.M. Kwak, F.J. Weissing and R. Bijlsma

Summary

In many plant species, pollen flow is mediated by insect pollinators. Changes in insect behaviour after fragmentation will have important consequences for gene flow. The isolating effect of distance and barriers were studied experimentally, using small patches of *Scabiosa columbaria*, which is mainly pollinated by bumblebees and syrphid flies. Patches were isolated by distances of 25m, 100m or 200m. A distance of 25m between patches already reduced pollen flow till 25%, while at a distance of 200m hardly any pollen grains were deposited. A steep decline with distance was found for both the transport of pollen grains and fluorescent dye powder. When a single pollen source was present the decline in pollen flow with distance was reflected in a reduction in seed set. As a result, isolated patches experienced either increased levels of intrapatch pollination or reduced seed set. Distance effects were consistent over fields and days. In contrast, the effects of a camouflage net, which was used to simulate a 'physical' barrier, were highly variable and no significant effects on the dispersal of pollen grains could be observed.

Introduction

Fragmentation of habitats leads to subdivision of large populations, severe reduction in population sizes and increasing isolation between the remaining populations. Such small remnant populations become vulnerable to environmental and demographic stochasticity, thereby increasing the risk of extinction (Soulé 1986; Caughley 1994; Schemske et al. 1994). Additionally, they may suffer from genetic erosion, because genetic processes, like genetic drift and inbreeding, become much more significant in small populations and can cause a reduction in mean fitness and adaptive potential of the populations, resulting in a further increase in their vulnerability to extinction (Barrett and Kohn 1991; Van Treuren et al. 1993b; Frankham and Ralls 1998; Bijlsma et al. 1999; Leijs et al. 1999; Bijlsma et al. 2000). The impact of such genetic risks strongly depends on the effective population size that can be significantly enlarged by gene flow between (sub)populations. Theoretically, the exchange of only one migrant per generation should be sufficient to effectively prevent significant genetic differentiation between subpopulations to occur, thereby reducing the impact of genetic decay (Hartl and Clark 1989; Ellstrand and Elam 1993; Scott Mills and Allendorf 1996). Gene flow is thus an important mechanism to limit the effects of genetic erosion. In this paper, we focus on the genetic effects of habitat fragmentation, by studying the amount of gene flow in relation to increased isolation between small subpopulations.

In many plant species gene flow occurs by both seeds and pollen (e.g. Rasmussen and Brødsgaard 1992; Poschlod et al. 1996; Hu and Ennos 1997; Latta et al. 1998). Seed dispersal offers an opportunity for colonisation of new habitats, but pollen generally disperses over larger distances. This study focuses on pollen flow in an insect-pollinated plant species, where the level of pollen flow is dependent on the flight patterns of its pollinators. Although pollinator species differ in the average flight distance between subsequent flower visits (see chapter 2), they usually commute between nearby flowers, which generally results in limited distances of pollen transfer (Pleasants 1991; Campbell and Dooley 1992; Morris et al. 1995). As a result, compared with wind-pollinated plant species, the potential for gene flow by pollen is rather limited. Indeed, studies using genetic markers regularly report very restricted genetic neighbourhoods for insect-pollinated plant species (Schaal 1980; Schmitt 1980; Handel 1982; Karron et al. 1995b). Because pollinator foraging is often geographically restricted, the visitation rate and frequency of pollinator flights between subpopulations will subsequently decrease with distance. Such a decline in pollinator flights between subpopulations results in a reduced gene flow by pollen, which in turn might result in an increased vulnerability for genetic erosion after habitat fragmentation.

As ongoing fragmentation naturally increases the distances between (sub)populations, it could lead to a reduction in gene flow (Campbell and Dooley 1992; Ouborg 1993a; Schittenhelm and Hoekstra 1995; Goodell et al. 1997; Richards et al. 1999). In large, unfragmented populations, pollen dispersal has been studied using a variety of methods, ranging from measurement of pollinator flight distance to analysis of the distribution of genetic markers (Schmitt 1980; Handel 1982; Karron et al. 1995b). In all cases, the observed pollen dispersal distances showed a leptokurtic distribution, with the bulk of dispersal events taking place within 10 to 20m, while dispersal over much larger distances was found to be rare. Although infrequent, pollen dispersal distances up to 1 km were reported in patchy populations (Ellstrand and Marshall 1985; Broyles et al. 1994; Stacy et al. 1996). However, distances of several kilometers between populations are often observed after habitat fragmentation. In combination with a strong leptokurtic pollen dispersal curve, such distances are expected to result in a significant increase in isolation between the remaining fragments, increasing their vulnerability to genetic erosion.

Additionally, habitat fragmentation and reductions in population size may also be accompanied by an increase in physical barriers, like hedgerows, roads and rivers, between the remnant (sub)populations. Several studies have shown that such barriers can significantly affect pollinator behaviour. Butterflies, for example, only migrated along rivers in southern Belgium and did not cross the area between different river basins, even when geographic distances between patches of suitable habitat were small (Nève et al. 1996). Another study confirmed the importance of vegetation structure for butterfly behaviour (Fry and Robson 1994). They showed that changes in the structure of the vegetation and especially the occurrence of boundaries between different vegetation types, drastically reduced the number of passages by butterflies. Other pollinator species, which preferably forage in open vegetation types, might be hampered by hedgerows or forests as well. Westerbergh and Saura (1994) speculated that the presence of spruce forest prevented bumblebee flights between more open areas, resulting in effective isolation of *Silene dioica* populations. In contrast, pollinator species inhabiting forests, might react to vegetation patterns by avoiding open areas. This was shown for males of four euglossine bee species that did not cross forest clearings (Powell and Powell 1987). Despite these effects of physical barriers on insect behaviour, to our knowledge, their consequences for pollen flow between plant populations have never been studied.

In this chapter, we study the importance of distance as well as barriers, for gene flow by pollen between subpopulations. In an earlier experiment, described in chapter 3, gene flow between patches of *Scabiosa columbaria* was observed to be significantly reduced by a distance of 65m. In the current experiments, distance between patches is increased to even larger distances, up to 200m, that, given the leptokurtic nature of the pollen dispersal curve, is expected to limit pollen flow extremely. Fluorescent dye powder was used to estimate pollen flow between patches. In the previous experiment, dye powder was shown to provide reasonable estimates of gene flow, compared to direct analysis of genetic markers (chapter 3). Additionally, direct deposition of pollen grains into emasculated patches is counted and related to dye powder dispersal, thus allowing to assess the reliability of different pollen flow estimates over larger distances. Isolation by a physical barrier is represented by a camouflage net, which reduced the visibility of neighbouring patches. The influence of this barrier for insect behaviour and its effects on pollen flow between patches are determined. By use of a similar experimental setup, the consequences of the barrier for gene flow can be compared with the effects of isolation by distance. The implications for genetic erosion after habitat fragmentation will be discussed.

Material and methods

Plant species

Scabiosa columbaria (Dipsacaceae) is a gynodioecious, protandrous perennial plant of dry calcareous grasslands, which is rare in The Netherlands (Van Treuren et al. 1991). The small tubular flowers are arranged in heads of 40-100 hermaphroditic flowers. Each flower is first male flowering for about two days, then enters a neuter phase of varying length. After all flowers have passed the male phase, the whole head enters the female phase. Usually heads are female for only a single day, but this can be extended to two days when the head remains unpollinated. *Scabiosa columbaria* has protruding anthers and stigmas and is dependent on insect pollinators for fertilization of the ovules. The species is self-compatible, and it has been observed that selfing reduces the percentage developed seeds (Van Treuren et al. 1993a).

Measurements

The experimental setup was analogous to the experiment of chapter 3, which will be used as a reference for gene flow estimates. Patches were located on adjacent mown hayfields situated in Assen, The Netherlands (52°59'N, 6°35'E). At the start of the experiments (between 10 and 11 AM) heads were virgin. Experiments lasted 5 to 6 hours, during which flowers in each patch were screened for the presence of insects. When walking along all patches, point observations were made with an interval of approximately 2 minutes. As in chapter 3, insects were classified into 6 groups, given in order of pollination efficiency (see chapter 2): Apidae (almost exclusively bumblebees); large syrphid flies (mainly *Eristalis* and *Helophilus* species, Diptera); other flies (Diptera, including the syrphid *Rhingia campestris*); *(Epi)Syrphus* species (Diptera); Lepidoptera and small *Siphona* flies (Diptera). Pollen flow was estimated based on direct pollen counts. This method gives quick results, is relatively cheap and proved to be useful for *S. columbaria* (chapter 3). At the end of the day the number of pollen grains (size 50-70 µm, pers. comm. G. Romeijn) was directly counted on all stigmas of female heads, using a hand-held lens of 20x. For each patch, pollen deposition was counted on all female heads. The experiments were repeated several times during the flowering season (August - October) to assess seasonal variation in visitor composition and weather conditions.

Isolation by distance

We used four linearly arranged patches, separated by distances of 25m, 75m or 100m (Figure 1). All patches were equally sized with respect to flower number and consisted of 20 ♂ heads and 10 ♀ heads. Only one patch contained functional male heads, producing pollen, and is designated source patch (S). On five of the 20 ♂ heads in the source patch, fluorescent dye powder (Radiant Color NV, Houthalen, Belgium) was applied as pollen analogue. In the other three patches, called receptor patches (R), the 20 'male' heads were either male sterile or were emasculated every 30 minutes and thus did not produce pollen during the experiment. One receptor patch (R1) was located at 25m of the source patch. Opposite from R1 the other two receptor patches were located at intervening distances of 100m, resulting in a distance to the source patch of 100m (R2) and 200m (R3) respectively (Table 1 and Figure 1, top and middle panel).

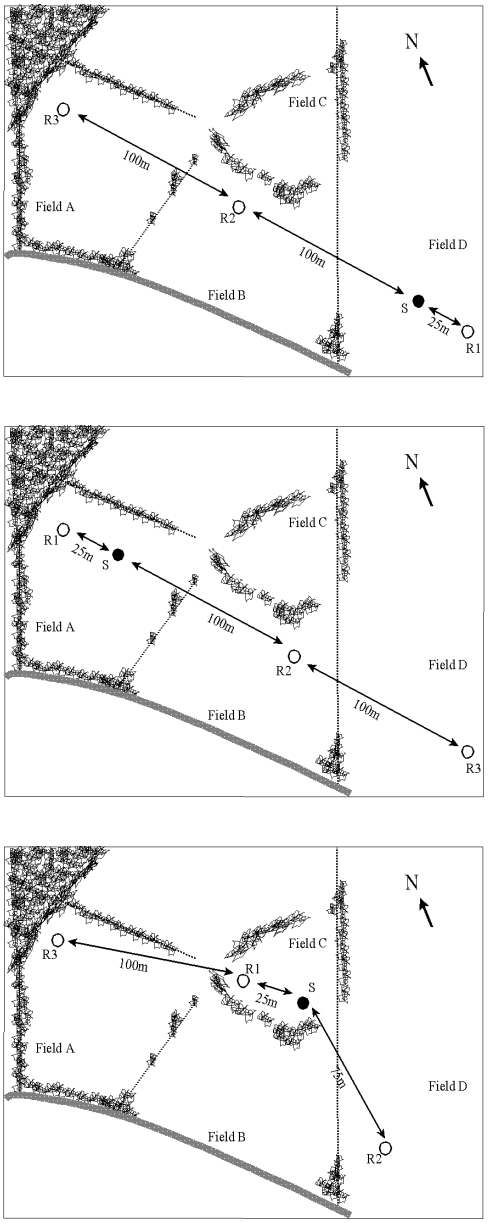

Figure 1 Location of the patches in the experimental fields for the isolation by distance experiments (source patch ●S, receptor patches oR). Top: on August 9, 12, 20, 27, patches were located at fields D,D,B,A. Middle: on September 5, 10, 18, patches were located at fields A,A,B,D. Bottom: on September 19, 30, patches were located at fields C,C,D,A and C,C,A respectively (see also Table 1). Bushes, ditches (dotted) and a small river (gray) are indicated.

Table 1 Details of the conditions in the isolation by distance experiments. Visitation was screened in 30 point observations per patch both in the morning and in the afternoon (September 30 afternoon only). Patches followed a linear arrangement: R1 - 25m - source - 100m - R2 - 100m - R3 (see text). Orientation is given from R1 to R3 as wind direction, location gives the meadows according to Figure 1. Each patch consisted of 20 ♂ and 10 ♀ flower heads. Emasculation of male flower heads was used in receptor patches only. MS: male sterile '♂' flower heads. R3*: location of R3 75m 'before' R1 (opposite of R2); distance to the source patch was 100m. **: R2 omitted and location of R3 75m 'before' R1, resulting in a distance of 100m to the source patch.

Emasculation	Day (1996)	Orientation	Location (R1,S,R2,R3)	'♂' in R3	Remarks	Duration	Sunshine (h)	Max. temperature (°C)	Wind speed (m/s) and direction
yes	August 12	SE - NW	D,D,B,A	♀	-	4 h	7.9	26.2	3 (ENE)
	September 5	NW - SE	A,A,B,D	MS	-	6.5 h	11.8	18.8	4 (NE)
	September 10	NW - SE	A,A,B,D	MS	-	7.5 h	1.0	17.2	4 (NW)
	September 18	NW - SE	A,A,B,D	MS	-	5 h	6.0	15.0	6 (E)
	September 19	SE - NW	C,C,D,A	MS	R3*	6 h	10.3	14.3	7 (NE)
	September 30	ESE - WNW	C,C,**,A	♂	no seeds	4.5 h	2.3	17.6	4 (variable)
no	August 9	SE - NW	D,D,B,A	♀	-	5 h	6.8	29.8	3 (SE)
	August 20	SE - NW	D,D,B,A	♂	-	5.5 h	13.3	31.7	5 (NE)
	August 27	SE - NW	D,D,B,A	♂	-	5 h	5.4	22.7	3 (variable)

Insect visitation was screened in two series of 30 point observations each during both the morning and afternoon (60 in total). These observations were made with approximately 2 minutes intervals. For logistic reasons insect visitation was not scored for receptor patch R3 (at 200m from the source). At the end of each daily experiment, the number of pollen grains was counted on all stigmas in each patch. The mean number of pollen grains per stigma per head was averaged over patches. In addition, ten stigmas per female head were sampled and mounted in glycerin on a microscope slide. The number of fluorescent dye particles per stigma was determined using a fluorescence microscope (400x). Small numbers of dye powder particles were counted (up to 16), larger numbers were classified into classes of exponent 2 (<32, <64, <128, ..., <2048 particles per stigma). The mean number of dye particles per stigma per patch was calculated using log-transformed numbers. Because the total number of dye powder particles applied and dispersed in the source patch differed considerably from day to day, dispersal to the receptor patches was presented relative to the amount deposited in the source patch to correct for these initial differences. When the ten stigmas had been sampled, the female heads were bagged to prevent further pollination. After ripening for about two weeks the seeds were collected and the percentage developed seeds was determined (Van Treuren et al. 1994).

The experiment was done on six days in 1996 (August 12 and September 5, 10, 18, 19, 30). Some modifications were unavoidable. On August 12, 30 ♀ heads were used for receptor patch R3 (at 200m), instead of 10 ♀ and 20 '♂' heads. For practical reasons, the number of pollen grains per stigma in the source patch was not counted, but estimated (see Effect of emasculation). On September 19 and 30, a different meadow had to be used due to farming practices, and receptor patch R3 was located in front of patch R1 (Figure 1 bottom panel). Thus, the experimental setup on these two days lacked a receptor patch at 200m from the source patch. On September 30, insect visitation was only observed in the afternoon.

Effect of emasculation

To investigate the consequences of emasculation on the attraction of flower visiting insects, the isolation by distance experiment was replicated three times with non-emasculated patches, on August 9, 20, 27 (Table 1 and Figure 1). All patches consisted of 20 intact male heads and 10 female heads, functioning both as source and receptor of pollen grains and fluorescent dye powder simultaneously. Five male heads in each patch received dye powder of a different colour, which was rotated between experimental days. Pollen deposition was not counted, but estimated in classes of 2.5 grains per stigma for each female head separately. Analysis of insect visitation, dye powder dispersal and seed production was as described before.

Isolation by barriers

Two replicates of three linearly arranged patches of *S. columbaria* were used. Replicates were located on different fields (Table 2, location of fields see Figure 1). All patches consisted of 20 ♂ heads and 10 ♀ heads and were separated by 25m grassland. The central patch functioned as donor of pollen and will be called source patch (S). The two peripheral patches were emasculated every 30 minutes and functioned as receptor patches. Physical isolation of one of the receptor patches was obtained by means of a camouflage net (double-folded army camouflage net), to mimic a hedgerow. The net was 1.8m high and placed exactly in between the source patch and the 'isolated' patch, perpendicular to the axis between the patches. In 1996 a net of 6m length was used, which was doubled in 1998 to 12m. During the afternoon 30 point observations per patch were made with intervals of 2 minutes to estimate insect visitation. At the end of each day, the number of pollen grains was counted on all stigmas for all ten female heads in each patch. The effect of the net on pollen flow was expressed as the ratio of the mean number of pollen grains deposited in this isolated patch (I) divided by the number deposited in the other receptor patch, without such a barrier ('non-isolated', NI). Effects of the barrier were tested for significance, using a Mann-Whitney-U test for differences in the mean number of pollen grains per stigma in isolated and non-isolated patches. The experiment was repeated several times during the flowering season (Table 2).

Viewed from the source patch the NI receptor patch was visible, while the I patch was hidden behind the camouflage net. Similarly, from the isolated patch the other patches could not be seen. Our expectation was that pollinators visiting the I patch would display less interpatch movements, and thus longer residence times, because they were not visibly distracted by the near presence of other patches. Therefore, we obtained additional information on insect behaviour during 1998 by recording the residence times (time between arrival at the first visited flower and leaving the patch) in all receptor patches and the behaviour of insects approaching the net during c. 2h per day. Due to logistic limitations, we only distinguished two groups of insects in the latter case: 'insects' (large Diptera and bumblebees) and butterflies.

Table 2 Details of the conditions in the barrier experiments. Visitation was screened during 30 point observations per patch in the afternoon. Patches were arranged linearly: non-isolated - 25m - source - 25m + net - isolated (see text, location meadows is given in Figure 1); their orientation is the same for both replicates and given from non-isolated to isolated as wind direction. Each patch consisted of 20 ♂ and 10 ♀ flower heads. Male flower heads were emasculated in the isolated and non-isolated patch. The camouflage net measured 6x1.8m in 1996 and 12x1.8m in 1998.

Year	Day	Orientation	Location replicates	Remarks	Duration (h)	Sunshine (h)	Max. temperature (°C)	Wind speed (m/s) and direction
1996	September 3	WNW – ESE	A,C	-	5.5	11.7	20.5	2 (variable)
	September 16	WNW – ESE	A,C	-	6	8.9	17.4	2 (variable)
	September 23	WNW – ESE	A,C	-	6.5	1.5	14.4	3 (variable)
1998	August 18	NW – SE	A,B	-	3.5	6.0	21.6	3 (N)
	August 31	NW – SE	A,B	-	3.5	7.3	18.9	3 (variable)
	September 7	NW – SE	A,B	-	4	2.6	20.5	3 (W)
	September 24	NW – SE	A,B	isolated patch first hour shaded	4	6.3	18.5	4 (E)

Results

Isolation by distance and effects of emasculation

Inherent to this kind of experiments, the variation between experimental days was high. Despite this variation, we pooled data from different days, searching for overall effects on pollination and seed set over a complete flowering season (see also Discussion). Insect visitation to emasculated patches was not affected by the distance to the nearest neighbouring patch, thus visitation to both receptor patches could be pooled (Figure 2A, compare R 25m and R 100m, χ^2=8.13, df=5, n.s.). The most common visitors were Diptera, mainly large syrphid flies and *Siphona*. However, for most insect groups, visitation was affected by emasculation (Figure 2A, compare S 0m with both R-patches). Overall, only Apidae (mainly *Bombus)* and *Siphona* did not significantly discriminate between patches in these isolation by distance experiments. All other visitors had a preference for the source patch (binomial proportion p>½, fly species P<0.01, butterflies P<0.05). In the experiments without emasculation, the composition of visitor species differed between the patches (χ^2=133, df=5, P<0.001, Figure 2C). The patch at 25m from the 'source' patch (S 25m) received relatively more visits by large syrphids and less visits by butterflies than S 0m, while the patch at 100m (S 100m) experienced the opposite visitation pattern. In the experiments without emasculation, all patches received more visits by Apidae and a lower frequency of *Siphona* visits compared to the experiments with emasculation (Figure 2, compare A and C). However, this was due to seasonal variation in insect abundance, since the difference is absent when species distributions were compared for the same period of the flowering season (Figure 2, compare B and C).

Pollen deposition declined with increasing distance to the source patch (Figure 3A). Already at 25m, the deposition of pollen grains is significantly lower than in the source patch and at 200m only few pollen grains were observed. In chapter 3, we demonstrated that pollination occurred mainly within the patch, and only few pollen grains originated from interpatch pollen transport. Therefore, we expected no differences in pollen deposition between the patches in the experiments without emasculation, where all patches functioned as pollen donor. However, as shown in Figure 3C, patches which were relatively isolated from each other (separated by at least 100m: S 100m and S 200m) showed a lower pollen deposition than the two relatively clustered patches (separated by only 25m: S 0m and S 25m). Seed production was also reduced by larger isolation distances, but less so than pollen deposition (Figure 3, A and B). Even at 200m some seeds were formed, and the difference in pollen deposition over 25m was not translated into differences in seed set. In the experiments without emasculation, all patches showed a similar seed production, despite the differences in pollen deposition (Figure 3, C and D).

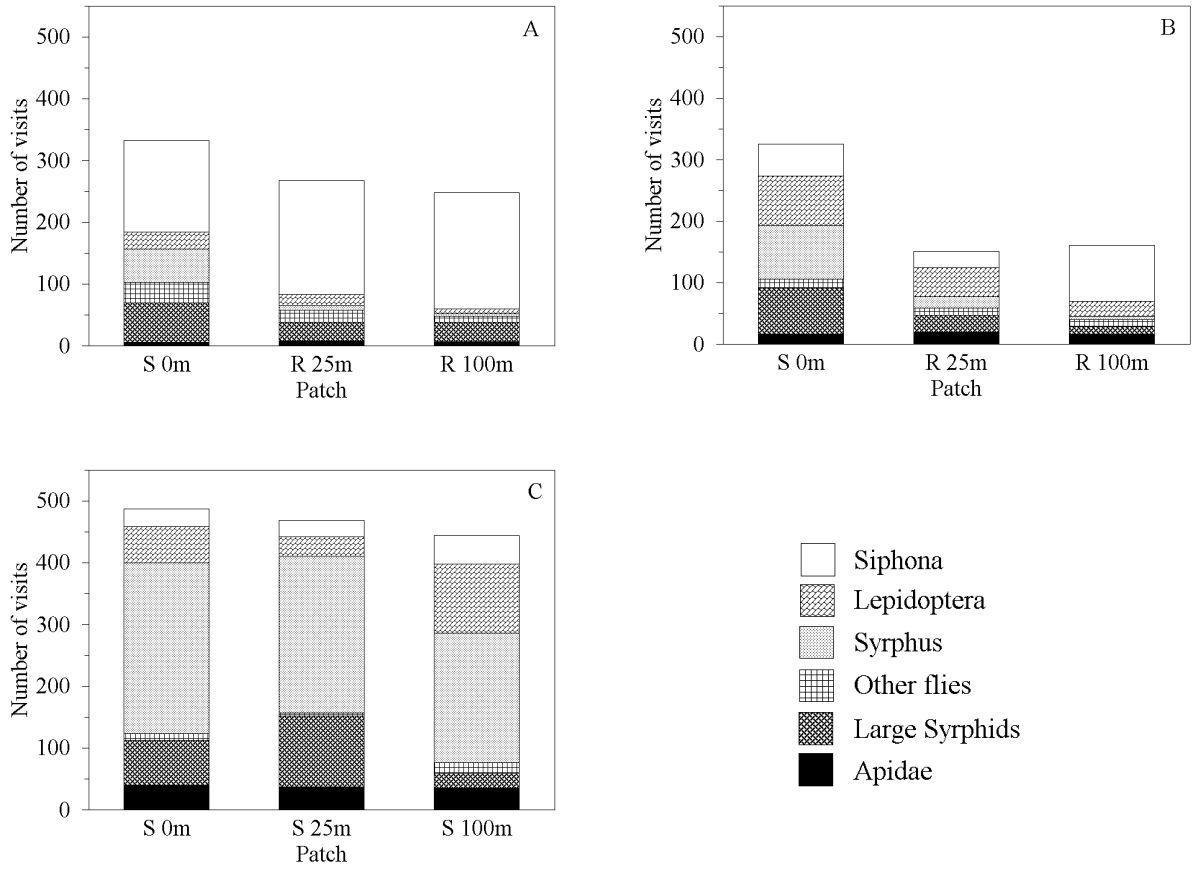

Figure 2 Mean visitation rate for patches at different distances to the nearest neighbouring patch (1996). The total number of visits per insect group during 60 point observations is given. (A) with emasculation of receptor patches: average values of all 6 days (August 12, September 5, 10, 18, 19, 30), (B) with emasculation of receptor patches: average values of 2 days (August 12, September 5), (C) without emasculation: average values of all 3 days (August 9, 20 and 27). Visitation was not determined for the receptor patch at 200m. S denotes a non-emasculated source patch, R denotes an emasculated receptor patch.

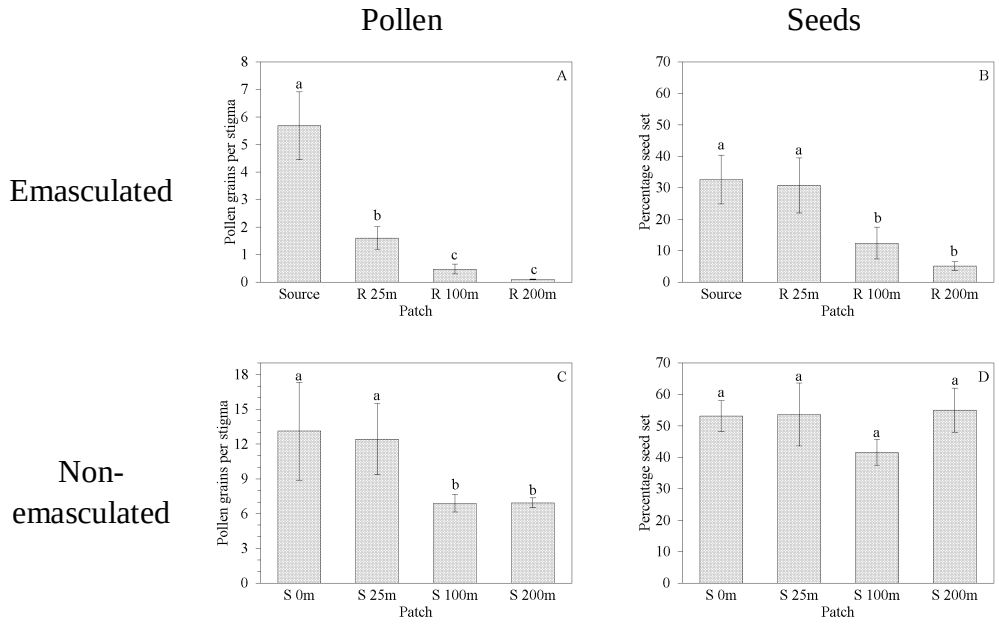

Figure 3 Pollen deposition (A,C) and seed set (B,D) in relation to isolation by distance in 1996. Mean values per patch are averaged over days (mean ± s.e.). Different letters indicate significant differences between patches at the 5% level (Kruskal-Wallis). Experiments with emasculated receptor patches were done on 6 days, and the number of pollen grains per stigma was obtained by direct counting (A). Experiments without emasculation were done on 3 days and pollen deposition was estimated in classes of 2.5 grains per stigma for each head (C). Note the different scales for the number of pollen grains per stigma.

Two estimation methods were used to measure pollen flow: dispersal of pollen grains and dispersal of fluorescent dye powder. The mean number of particles found per stigma per patch was expressed relative to the number found in the source patch, for both pollen grains as well as dye powder (means back-transformed from logarithms to numbers). Dispersal of dye powder at September 19 is excluded from the analysis, because dispersal and deposition were extremely low on this day, even in the source patch, making the deposition ratios for this day very sensitive to small variations and measurement errors. Independent of the estimation method, dispersal declined steeply with increasing distance (Figure 4). At a distance of 25m, deposition of pollen and dye powder dropped below 30% of the amount observed in the source patch. Emasculation had no effect on the dispersal of dye powder, since the experiments with and without emasculation resulted in identical dispersal curves for dye powder. However, dispersal of pollen grains, which was only measured under emasculated conditions, differed from the dispersal of dye powder (Figure 4). At 25m, deposition of dye powder was reduced to 10%, while the deposition of pollen grains was still 28% (t-test, $P<0.05$). Also at 100m, a higher deposition of pollen grains than dye powder was observed (t-test, $P<0.05$). The decrease with distance was thus more severe for dye powder than for pollen grains. At larger distances only marginal deposition of both pollen grains and dye powder occurred. Therefore, we conclude that distance has a strong isolating effect on both pollen flow and dye powder dispersal between patches of *S. columbaria*.

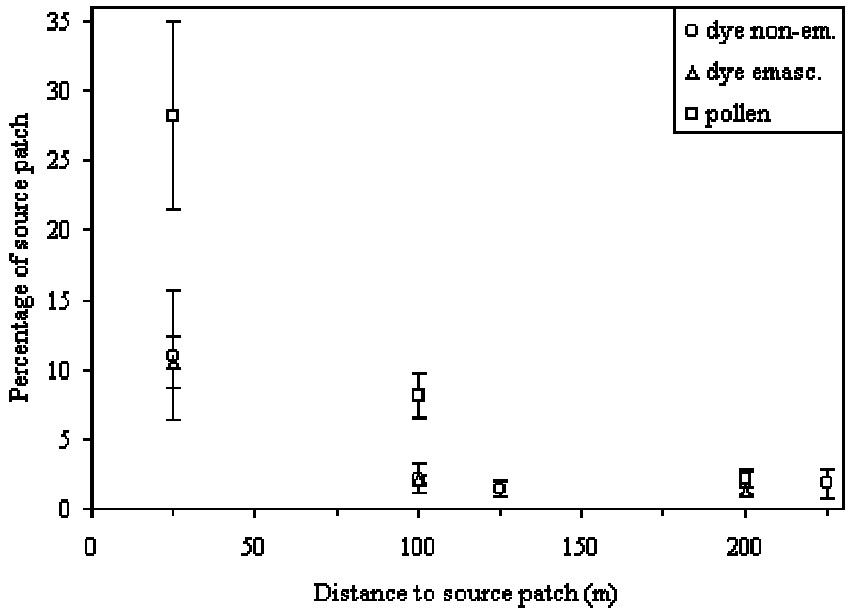

Figure 4 Deposition of pollen and fluorescent dye powder as a function of distance. The mean number of particles per stigma per patch is expressed as percentage of the number found in the source patch. Dispersal of dye powder and pollen grains into emasculated patches is averaged over five days (see text). Dye powder dispersal in experiments with non-emasculated patches is averaged over all three days (mean ± s.e.).

Isolation by barriers

An increase in distance between patches thus resulted in a severe decline in pollen flow. By means of a camouflage net placed between two patches, it was tested whether a physical barrier had the same effect as isolation by distance. The experiment was repeated in two years, 1996 and 1998. Between years, there was substantial variation in insect species composition. In 1996, the visitation rate was about 6.5 times as large as in 1998. This difference resulted mainly from *Siphona* flies, visiting the patches in huge numbers in 1996, but virtually absent in 1998: 125 visits per patch during 30 observation rounds in 1996 and only 1 visit per patch in 1998. Although the composition of the species varied considerably, total visitation by important pollinators (Apidae, large syrphids and other flies, see chapter 2) was relatively similar between years. Therefore, only visitation by these important pollinators is presented in Figure 5. For these insect species, composition was found to be significantly different for I and NI patches (overall, χ^2=12.1, df=2, P<0.005), with I patches receiving more visits from bumblebees and large syrphids and less visits from other fly species. However, caution is called for as visitation patterns varied greatly between fields, days and years, leading to the conclusion that the camouflage net did not consistently affect pollinator species composition. In addition, although some differences in residence time were observed between I and NI patches (Table 3), these were highly variable, both in time and space and the differences were clearly not consistent. Furthermore, direct observations of insect behaviour when approaching the net, revealed that, although many insects changed flight directions at the net, a similar number simply passed it, mostly by flying over it. The fraction of insects that effectively changed flight direction when approaching the net was on average (mean ± s.e.): 0.58 ± 0.03 (n=316) in 1996 and only 0.36 ± 0.06 (n=56) in 1998, respectively. For butterflies, the net was slightly more effective: the fraction that was 'blocked' was 0.88 ± 0.07 (n=25) in 1996 and 0.54 ± 0.10 (n=24) in 1998.

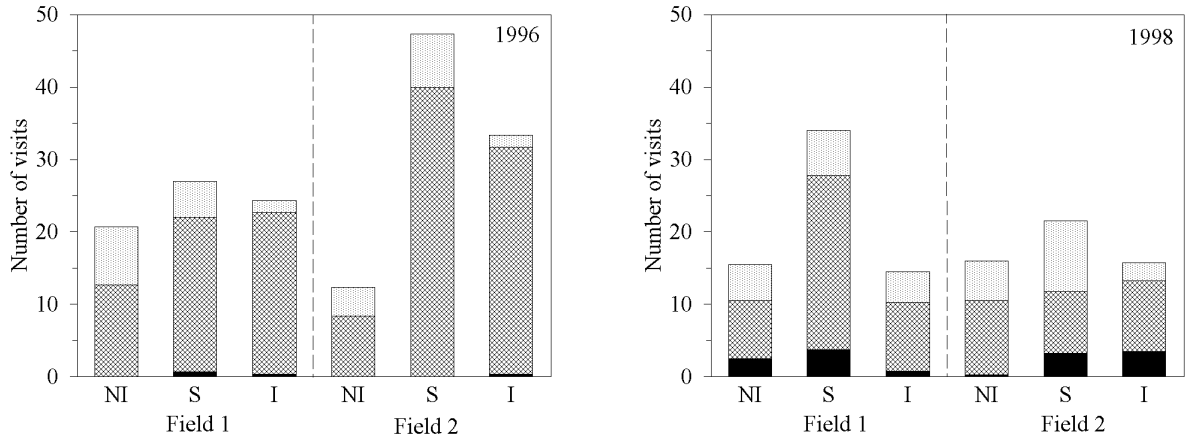

Figure 5 The number of visits per insect group for patches that are isolated by a barrier (only important pollinators are shown). For both fields, the total number of insects per patch during 30 point observations in the afternoon is averaged over 3 days in 1996 and 4 days in 1998, respectively. The barrier was positioned in between the source patch S and the isolated receptor patch I (see text). As a control for effects of distance, a non-isolated patch NI was used. Location of patches and fields is given in Table 2 and Figure 1. 1996: net length 6m; 1998: net length 12m. Filled: Apidae; Hatched: Large Syrphids; Dotted: Other flies.

Table 3 Influence of the camouflage net on residence times of large syrphid flies in 1998 (seconds, mean ± s.e.). Sample sizes are given in brackets. X excluded, because sample size was <5.

Date	Field 1		Field 2	
	Non-isolated	Isolated	Non-isolated	Isolated
August 31	308 ± 148.9 (5)	301 ± 210.9 (7)	X	149 ± 61,0 (6)
September 7	73 ± 46.3 (5)	X	202 ± 53.9 (17)	233 ± 52.0 (26)
September 24	181 ± 81.4 (9)	78.3 ± 33.7 (9)	135 ± 23.6 (28)	146 ± 42.9 (22)

The foregoing indicates that the camouflage net did not act as a barrier. This ineffectiveness is reflected by its consequences for pollen flow. Pollen deposition in the receptor patches (I and NI) ranged between 0.8 and 4.7 pollen grains per stigma. When the number of pollen grains in the I patch is expressed as fraction of the number found in the NI patch (pollen deposition ratio), the effect of the camouflage net appeared non-significant and highly variable (Figure 6). In 1996 a tendency for reduced pollen flow to I patches was found in 4 out of 6 replicate experiments, but the only significant effect of the camouflage net showed an increased pollen flow for the I patches. In 1998 the camouflage net was twice as large, but pollen flow was again not reduced. Out of 8 replicates, only two gave a significant difference between I and NI patches and both showed an increase in pollen flow to I patches. In conclusion, the camouflage net clearly did not function as a barrier for pollinating insects and did not limit pollen flow.

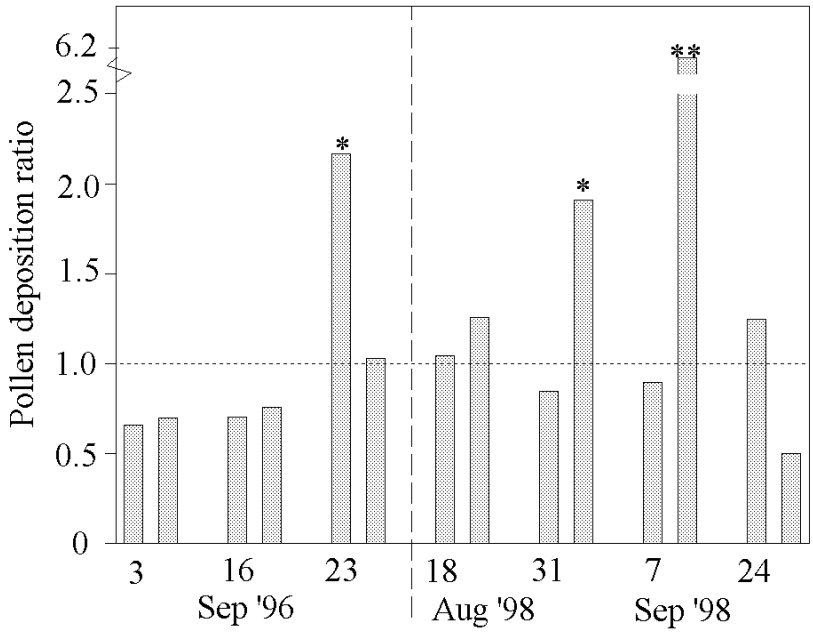

Figure 6 Effects of the camouflage net on pollen flow. The mean number of pollen grains per stigma in the isolated patch is given as fraction of the number of pollen grains found in the non-isolated patch. A ratio below 1 indicates a decrease in pollen flow to the I patch, while a ratio above 1 indicates an increase in pollen flow to the I patch. For each day the left bar gives results for field 1, the right one for field 2. Differences in mean pollen deposition between the isolated and non-isolated patch were tested non-parametrically. Significant differences at the 5% level are indicated with *, at the 1% level with ** (Mann-Whitney U test).

Discussion

Overall, we observed large variation in the results between both days and fields, which may have greatly hampered the repeatability of the experiments. Several factors may be responsible for this large variation. Changes in abundance and species composition over the flowering season and between different fields were found (Figure 2 and 5). Such variation in visitation results in a pollinator guild with varying characteristics (see chapter 2), which will influence pollen dispersal. Additional variation in visitation and pollen flow between patches, fields and days might be related to wind direction, the location of ‘sources’ of pollinating insects (nests, other flowering areas, etc.) or random variation in the body load of visitors, depending on their individual foraging history. Since recapture of marked syrphid flies occurs infrequently (own data, Ottenheim pers. comm.), we were not able to track individual foraging patterns and could not determine how many individual insects were responsible for visitation of *S. columbaria*. Studies with individually marked bumblebees visiting other plant species, showed that high visitation rates might be due to a rather restricted number of individuals (Thomson 1996; Velterop and Kwak 1997; Kwak et al. 1998). In such cases, the flight pattern of those individuals will be crucial for patterns of pollen flow and might be highly variable. Part of the observed variation may also be due to correlations between observations. For example, when a pollinator visits a female head, several stigmas receive pollen grains, resulting in correlated numbers of pollen grains per stigma among stigmas within the head. On a larger scale, correlations between heads within the same patch may develop, since insects often visited more than one head before leaving a patch. Thus, visits to heads within a patch are not independent,

but correlated as a consequence of the foraging behaviour of individual pollinators. Stacy et al. (1996) found such a correlation for the frequency of outcrossing within patches of tropical trees. These sources of variation are inherent to the type of experiments, using (semi-)natural conditions. By combining the results of several replicates, we believe to get an overall view of the pattern of pollen flow over the flowering season. Because daily experiments were spread over the season, temporal variation is included in our estimate of pollen dispersal. Although pooling of the data is statistically not allowed, we think that a sound conclusion can be reached (see also Figure 7).

We found a clear effect of distance between patches on pollen dispersal. Pollen deposition declined severely with increasing distance to the source patch (Figure 3). Small differences in pollination intensity will not be reflected in seed set, since maximal seed set is reached for approximately 4 pollen grains per stigma (chapter 3). Nevertheless, seed set obviously also decreases with distance. At 200m, only one seed per head is produced on average. For self-incompatible plant species, such a large decrease in pollen flow will imply a shortage of compatible pollen types and consequently a severe reduction in seed production. For *S. columbaria*, a self-compatible species, high intrapatch pollen deposition usually weakens the effects of distance on seed production, as observed in the experiment without emasculation. Still seed quality may be reduced when intrapatch pollination is high, but this was not studied.

Despite the generally high intrapatch pollination (see also chapter 3), pollen deposition was lower in the relatively isolated patches at 100m in the distance experiment without emasculation (Figure 3). This may be due to the lower visitation by large syrphid flies (Figure 2C), which are important pollinators of *S. columbaria* (chapter 2; Kwak and Velterop 1997). The reason for this lower visitation rate is unknown, but it may easily result in fewer movements between male and female heads. It is, however, unlikely that the lower visitation results from a distance effect *per se* or from a location effect, since it was not found in the setup with emasculated patches. Emasculation affects visitation patterns of most insect groups, including all syrphid flies (Figure 2A). Especially the preference for the pollen-containing source patch of large syrphids may have aggravated the decline in pollen flow with distance to the source patch.

Both measures of pollen flow show a steep decline with distance, although dispersal of pollen grains declines less steep with distance than dispersal of fluorescent dye powder (Figure 4). The same difference in dispersal characteristics is observed in the experiments described in chapter 3. The discrepancy may be caused by differences in size between pollen grains and dye powder particles (50-70 µm resp. 5 µm), or variation in the absolute amounts available for dispersal. Alternatively, dispersal of pollen grains and dye powder might be affected differently by emasculation, as reported in a review by Snow et al. (1996). Gene flow by pollen will only be effective if the transported pollen grains are successful in fertilization of ovules. In a comparative experiment, we investigated the dispersal of fluorescent dye powder in comparison to effective gene flow of allozyme markers in a slightly different setup (chapter 3). Despite substantial variation between days, both methods show similar dispersal patterns, when averaged over several replicates (Figure 7, chapter 3). Thus, dye powders give reliable estimates, while pollen counts in emasculated patches slightly overestimate the amount of effective gene flow between patches. If only relative effects on pollen flow are important, both measures are useful to compare experimental treatments.

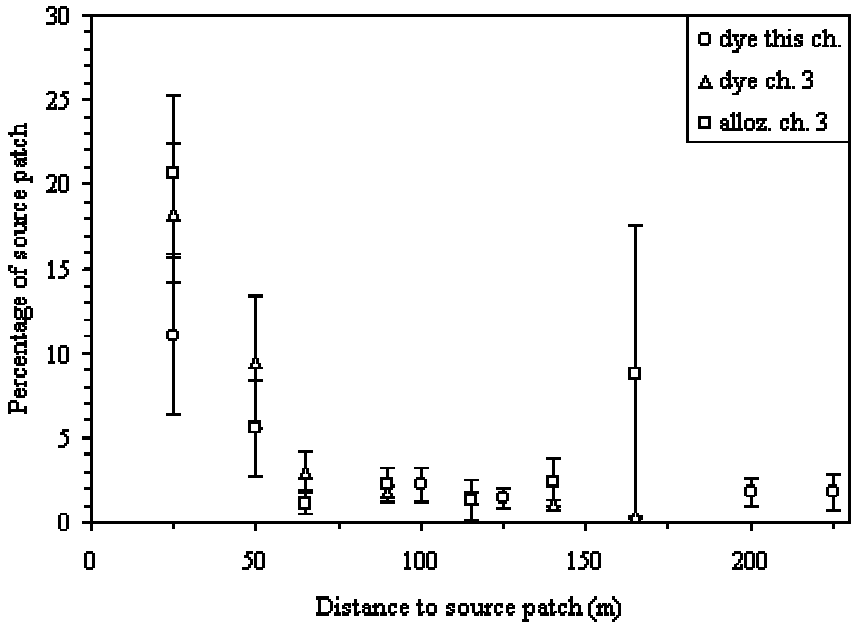

Figure 7 Deposition of fluorescent dye powder and allozyme markers as a function of distance in 1996. At each distance, deposition is expressed as percentage of the deposition found in the source patch and averaged over days (mean ± s.e.). This figure combines data from two different experiments. Dispersal of dye powder over bare grassland, without intervening patches, was determined in five daily experiments, as described in this chapter. In a different set of experiments, dispersal was determined between patches separated by grassland with intervening patches of flowering *Scabiosa columbaria* (see chapter 3). Dispersal of dye powder and allozyme marker alleles was simultaneously determined on five different days. The huge standard error of allozymes at 165m is due to exceptionally high gene flow at a single day.

In Figure 7, we combine the results of this chapter and chapter 3, to evaluate the effects of intervening patches on pollen dispersal distances. We anticipated differences in dispersal patterns, because other studies reported an increase in mean dispersal distance with increasing distance between (clumps of) plants (Manasse 1992; Morris 1993). In contrast, we found almost identical dispersal patterns for patches separated by large distances of grassland (this chapter) or several linearly arranged patches at smaller distances (chapter 3). Apparently, the 'intermediate' patches of chapter 3 have no net effect on pollen dispersal distance, although they could have functioned as 'stepping stones' for the pollinating insects. In principal, Apidae are capable of flying several hundreds of meters (e.g. Kwak et al. 1998) and flies are potentially strong flyers too (50 km/week, Banziger 1996). Nevertheless, regular pollen flow appears to be restricted to distances less than 100m. Dispersal over larger distances may be too infrequent to detect in our experiments, although its impact on the genetic population structure can be substantial (Hartl and Clark 1989; Levin 1995).

We used a camouflage net to represent a physical barrier between patches of *S. columbaria*. The results were highly variable. Often, a tendency for reduced pollen flow to I patches was found, but in all significant cases higher pollen flow to I patches than to NI patches was observed. This may be due to the limited height of the net or to a few individual pollinators with a large body load, pollinating many flowers during a single visit to a patch (see above). The results of this experiment are very inconclusive and it has to be repeated in an improved setup, e.g. using a higher camouflage net or alternative structure as barrier to insect movement.

The strong leptokurtic distribution of pollen dispersal distances results in a strong limitation of pollen and gene flow in relation to distance. Pollen grains barely disperse over distances larger than 200m, thus (sub)populations separated by larger distances are effectively isolated. In The Netherlands, the distance between populations of *S. columbaria* is at least 850m, but generally several kilometers (Ouborg 1993a), preventing gene flow by pollen. As seed dispersal is also very restricted (given current management practices, Fischer et al. 1996), Dutch populations of *S. columbaria* are thus effectively isolated from each other, making them vulnerable to genetic erosion.

Acknowledgements
We thank the students of the course of General Ecology (September 1996, 1998) for help with the observations of insect behaviour, Arjen van ‘t Hof for counting dispersal of fluorescent dye powder and W. van Delden and J. van Andel for critically reading the manuscript. We also thank Joop, Iris and Marije Smittenberg for their hospitality during many long days of fieldwork and Mr. and Mrs. Meyeringh and Mr. Smittenberg for use of their grasslands.

Do corridors increase pollen flow between patches of *Scabiosa columbaria* (Dipsacaceae)?

With M.M. Kwak and J. van Andel

Summary

Pollen flow between artificial patches of *Scabiosa columbaria*, mainly pollinated by syrphids, was studied in a semi-natural environment. Three linearly arranged patches of 30 heads each were used. The central patch served both as pollen donor and receptor patch (complete flowers), the other two patches served only as receptor patches (emasculated flowers and female flowers). The central patch was connected over a distance of 25m with one receptor patch by a corridor of flowers. The plant species of the corridor varied: either *S. columbaria* female heads, *S. columbaria* male heads, *Origanum vulgare* inflorescences or *Aster multiflorus* heads were used. The corridor species shared visitors with *S. columbaria*. The second receptor patch was not connected with the donor patch and served as a control. Experiments were performed with different arrangements of the corridor. The presence of a corridor did not influence the total insect visitation per patch. Syrphid species did not differ in hop size (distance between two subsequently visited heads) if the corridor consisted of *S. columbaria* female heads. In the *Aster* corridor syrphid species made larger hops than in the *Scabiosa* corridor. In the *Aster* corridor hop size of *E. arbustorum* differed significantly from the other two insect species. All together, insects flew larger distances between subsequently visited heads if the corridor contained *Aster* heads than when the corridor contained *S. columbaria* heads.

Despite the fact that a fair amount of pollen was deposited on the female heads in the corridor, pollen flow was increased significantly in 4 out of 10 experiments if the receptor patch was connected with a corridor containing *S. columbaria* male or female heads. In only one experiment a significant decrease in pollen deposition in the connected patch (connected with a female *S. columbaria* corridor) was observed. Pollen flow was significantly reduced in 3 out of 12 experiments with *Aster* or *Origanum* as corridor species and was increased significantly only once. A significant increase of heterospecific pollen deposition was observed if the corridor species was not conspecific.

Potential seed set (based on the number of stigmas with four or more *S. columbaria* pollen grains) showed the same overall pattern as mean number of pollen deposited, although the ratios in pollen deposition and potential seed set differed largely between experiments.

Introduction

Population genetic theory predicts that, as a consequence of genetic drift and inbreeding, small and isolated populations will have decreased levels of genetic variation. Even favourable alleles may be lost and the potential to adapt to a changing environment may be seriously diminished (Vrijenhoek 1985). This genetic erosion may lead to reduced fitness of individuals in a population and will ultimately increase the risk of extinction of populations and of species. However, the deleterious effects of genetic erosion can be counteracted by gene flow between populations. In plants, pollen flow in particular contributes to preservation of genetic variation. As a rule of thumb, the exchange of one migrating individual per generation should be sufficient to prevent genetic differentiation between subpopulations (Ellstrand and Elam 1993; Scott Mills and Allendorf 1996). Thus, management practices that prevent further isolation of plant populations or that increase pollen flow between populations or patches within a population may be important for genetic variation in small populations. Levin (1995) discussed the possible positive function of outliers, scattered plants, often occurring hundreds of meters or several kilometers beyond the limits of local populations. Outliers may form large, diffuse assemblages of interbreeding plants that fill gaps between local populations.

The hypothesis that habitat corridors or stepping-stones may increase migration of animals between populations is generally accepted (Opdam et al. 1993; Beier and Noss 1998). Several studies provided persuasive data regarding the utility of corridors, but few studies actually test the impact of the presence of a corridor on the mobility of animals and the value of these exchanges for the viability of the animal population (see review of Beier & Noss 1998). According to these authors, birds and mammals are target species in most studies and studies on insects are scarce with the exception of ground beetles (Vermeulen 1994). In the case of flower-visiting insects, visiting flowers in a corridor, we hypothesize that these movements may increase their foraging area, which has also an impact on the gene flow and seed set of plants. Restricted movements of flower-visiting insects will result in restricted pollen and gene flow of the plants. Thus, we need to know how flower-visiting insects are influenced not only by the arrangement of their food plants, but also by other characters of the landscape like the presence of bushes, forest, waterways etc. Fry & Robson (1994) found that hedgerows acted as barriers for butterflies. Powell & Powell (1987) found that males of four euglossine bee species did not cross 100m pasture clearings from continuous forest to forest fragments, even though bees can fly more than 20km per day from a release point (Janzen 1971). In contrast, Sutcliffe & Thomas (1996) found that open, grassy tracks were important as corridor for butterflies, acting as conduits between fields and glades.

The impact of the presence of a flower corridor on pollen flow of plants is the issue of this chapter. The consequences for the viability of insect populations of an increasing exchange among flower-visiting insects are left out of consideration. If an insect visits several patches of a target plant species within a larger area which contains flowers (same or different flowering species), these intervening flowers may serve as bridge or corridor. They may even become part of a fixed foraging route of flower visitors, for instance traplining as was found for bumblebees and euglossine bees (Janzen 1971; Heinrich 1975, 1976; Thomson et al. 1997; Williams and Thomson 1998; Comba 1999).

Three possibilities, not mutually exclusive, can be distinguished concerning the effects of a flower corridor on the amount of pollen exchange between two patches: 1. Pollen exchange is zero, although the patches may be within the possible action radius of the pollinators because each patch has its own pollinator guild; 2. Pollen exchange occurs if visitors cross the distance between two patches without loss of pollen because they are not visiting intervening flowers of the same or other species (guidance effect); 3. Pollen exchange occurs but, due to visits to the intervening flowers (guidance effect), pollen is lost. In the last case, pollen exchange between patches is dependent on the amount of switching of insects between the patches and the loss rate of pollen in the corridor. Loss rate in turn is dependent on the length (both in meters and in number of flowers) and on the plant species in the corridor. Visitation of another plant species forming the corridor may result in heterospecific pollen deposition in the connected patches.

In this chapter we present data on pollen flow between patches of *Scabiosa columbaria*, a species pollinated by syrphid flies and bumblebees in The Netherlands (Kwak and Velterop 1997). Artificial patches were connected with a corridor of the same plant species between a pollen source patch (either male or female heads as corridor species expecting a guidance effect and in the female corridor also a loss of pollen) and a receptor patch. As control, artificial patches not connected with a corridor were created. Also experiments were done with another flowering plant species in the corridor. A possible guidance effect with a pollen loss in the corridor and the chance of heterospecific pollen deposition in the receptor patch were expected. Pollen deposition in connected patches was compared with pollen deposition in control patches without a corridor. This study is part of a larger project in which the consequences of population fragmentation for pollen and gene flow by insects are studied in experimental situations in the field.

Material and methods

Scabiosa columbaria L. (Small scabious, Dipsacaceae), a perennial, outbreeding plant species, is rare in The Netherlands. It occurs in dry grassy places on calcareous soils. The small, blue-violet, tubular flowers are arranged in heads (30-100 flowers per head). Each flower is first male for about two days, then enters a neuter phase of varying length. After all flowers have passed the male phase, the whole head is female for about one day. Both anthers and stigmas protrude out of the flower and are easily touched by various species of insects.

In total, 22 experiments were conducted in unfertilized hay fields in the North of The Netherlands (Assen, 52 °59'N, 6°35'E) in 1996 and 1997, from August till early October. Experiments were done at four neighbouring fields A (fietspad), B (boomstronk), C (bessenland) and D (buurman). Per day one or two experiments were conducted. Each experiment consisted of three patches arranged in a line (WNW-ESE orientated) with an interpatch distance of 25m. The distance of 25 meter between patches was chosen because earlier experiments (chapter 4) demonstrated that over this distance pollen flow from donor towards the receptor patch was reduced to about 25%. Moreover, the size of the fields did not permit much larger distances between patches. Each patch contained 10 female (virgin at the start of the experiment) and 20 male heads. Plants in pots were used. Only the central patch was allowed to deliver pollen and this patch served both as donor and receptor patch. The male flowers in the two distant patches were emasculated every half an hour, since the development of anthers continued during the day (see chapter 2). The central patch and one of the distant patches were connected with a corridor. This corridor contained either 30 female (virgin at the start of the experiment) *S. columbaria* heads, or 30 male (but emasculated) *S. columbaria* heads or 30 *Aster multiflorus* cv blue star heads or 30 Origanum *vulgare* inflorescences. *Origanum* was chosen since in natural populations of *S. columbaria*, insects often carried a pollen load that contained a large number of *Origanum* pollen (Kwak and Velterop 1997). Heads of *Aster* were chosen because their morphology enables the same group of insects to visit them and they were available later in the season when *Origanum* was out of flower.

The arrangement of heads or inflorescences within the linear corridor was either regular with a distance of about 80 cm between the individual heads or they were grouped in 6 clusters of each five heads or five inflorescences, at a distance of 1.5 m between each other. The position of the corridor was either parallel, along an imaginary axis connecting two patches or across this imaginary axis between the patches. Table 1 summarizes details of the experiments, performed on several census dates.

Table 1 Conditions during pollination experiments with corridors between patches of *Scabiosa columbaria* in 1996 and 1997. * Corridor direction reversed compared to all other experiments. Pollinating insects: Diptera, mainly Syrphidae and Apidae; *Siphona geniculata* and Lepidoptera excluded. Number of pollinating insects given per 60 observation surveys in the donor patch.

Arrangement	Corridor species	Date	Field	Mean and (max) day temperature (^{O} C)	Wind direction	Wind speed (m/s)	Duration experiments (h)	Observations on insect hop size	Number of pollinating insects	Bar letter in Figure 1
parallel, regular	*Scabiosa* female	Sept 1, 1997	A, C	18.6 (23.2)	SW	3	5.5	yes	207, 126	a,b
	Scabiosa female	Sept 11, 1997	A	13.2 (18.1)	E-SE-S	3	5	no	176	c
	Scabiosa female	Sept 29, 1997	B, C	12.1 (17.5)	variable	2	4.5	yes	99, 69	d,e
	Scabiosa male	Sept 18, 1997	B, C	12.1 (17.7)	E-NE	2	5.5	yes	84, 81	f,g
	Scabiosa male	Sept 23, 1997	B, C	10.7 (17.4)	NE-SW	2	4	no	33, 123	h,i
	Aster	Sept 8, 1997	A, C	15.9 (17.3)	SW	6	5	no	80, 44	k,l
	Aster	Sept 11, 1997	C	13.2 (18.1)	E-SE-S	3	5	no	120	m
	Aster	Sept 25, 1997	B, C	12.6 (20.5)	SW	2	5.5	yes	89, 72	n, o
parallel, clusters	*Scabiosa* male	Oct 7, 1996	C*	9.6 (15.9)	variable	3	5	no	245	j
	Origanum	Aug 8, 1996	D	16.9 (23.4)	variable	3	5	no	112	p
	Origanum	Aug 14, 1996	B	17.8 (22.6)	variable	4	5.5	no	103	q
	Aster	Sept 9, 1996	A, B	13.0 (20.2)	variable	2	6	no	82, 150	r, s
across, clusters	*Origanum*	Aug 21, 1996	D	19.9 (25.7)	variable	3	5	no	201	t
	Origanum	26 Aug 1996	D	15.5 (22.0)	SW	3	6	no	292	u
	Origanum	4 Sept 1996	B	14.5 (18.5)	variable	3	5	no	130	v

The experiments lasted between 4 and 6 hours. During the day, heads in each patch and in the corridors were screened for the presence of naturally occurring insects (no attempt was made to introduce particular insect species). This screening, called surveys, lasted about 2 minutes for the three patches and the corridor; screening was conducted 15-60 times per day. Insect species were classified as syrphids (most common species *Eristalis tenax, Eristalis pertinax, Eristalis intricarius, Eristalis arbustorum, Eristalis nemorum, Helophilus pendulus*), flies, small flies (most probably *Siphona geniculata*), bumblebees (*Bombus pascuorum* Scop., mainly males and *Bombus terrestris* L.) and butterflies (*Autographa gamma, Thymelicus* spec., *Inachis io, Pieris rapae*). All insect species are (very) common species in The Netherlands. Visitation is given as the number of observed visits per 60 surveys per observation day.

In order to understand the results of pollen deposition, we followed foraging syrphids, visiting heads in the corridor. Each switch between heads in the corridor is called a hop and the distance between two subsequently visited heads was noted as hop size. Minimum hop size was 1 (see also Figure 2). The total number of observations of hop sizes per individual insect was called a bout. Hop sizes per insect species per corridor type and mean hop size per corridor type were calculated. Only bouts with a minimum of 3 hops (4 observed visits) were included.

At the end of each experiment, female heads were collected and the number of *S. columbaria* pollen grains per stigma was counted with a hand-lens of 20x. Either all stigmas or a random sample of 10-15 stigmas were counted and the mean deposition per stigma per head was calculated. After the direct counting of *S. columbaria* pollen in the experiments with *Aster* as corridor species, stigmas of all female heads in the receptor patches were cleaned with a piece of a sticky gel (Beattie 1972). The numbers of *S. columbaria* and *Aster* pollen per head were counted in this gel using a microscope and the fraction *Aster* pollen was calculated.

Effects of the corridors were tested for significance of the differences in the mean number of pollen grains per stigma, deposited in the connected and control patch, using a t-test or Mann-Whitney U test.

Results

Visitation

Visitation showed large variation; in some experiments very large numbers of insects visited the heads (experiments j and s in Figure 1, note the difference in scale of the Y-axis between the figures) while in others less than 50 efficient pollinators were recorded (species of Apidae and Diptera, the small fly species *S. geniculata* excluded): experiments h and l in Figure 1. Most frequent visitors on nearly all days were syrphid flies, especially the species *Eristalis tenax, Eristalis arbustorum* and *Helophilus pendulus*. Apidae, mainly bumblebees, were present in reasonable numbers in the experiments done with *Origanum* as corridor species. Butterflies were also regular visitors in these experiments, both on *Scabiosa* and *Origanum*. The small fly species *S. geniculata* was sometimes present in very large numbers, particularly in the experiments with *Aster* as corridor species.

In summary, *S. columbaria*, *Aster* and *Origanum* shared the same visitor species. Thus visitors of the donor patches of *S. columbaria* may use the corridor species in order to reach the other *S. columbaria* patch.

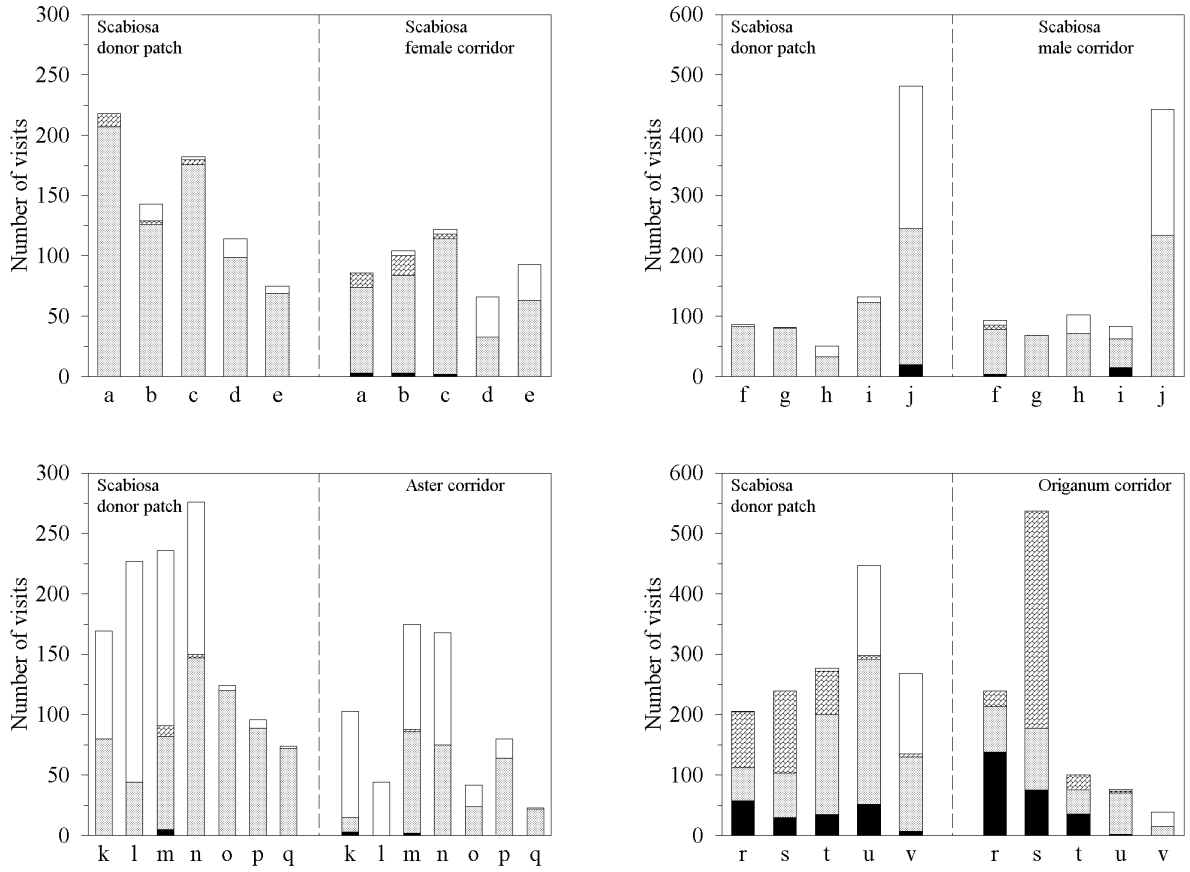

Figure 1. Frequency of insect visits to the donor patch of *Scabiosa columbaria* and the corridor consisting of various plant species, during 60 observation surveys during the day. Letters on X-axis indicate the date and field characteristics mentioned in Table 1. Note the different scales of Y-axes.

Insect visitation of the corridor

Some examples of long visitation bouts of individual syrphids in the corridor are given in Figure 2. Although the mean number of hops in a bout of an insect visiting an *Aster* corridor (5.3 ± 2.6, mean ± s.e.) was shorter than in the *Scabiosa* corridors (*Scabiosa* female and *Scabiosa* male, all regularly arranged, 6.8 ± 4.6, see Table 1), this difference was not significant (Oneway Anova P>0.05).

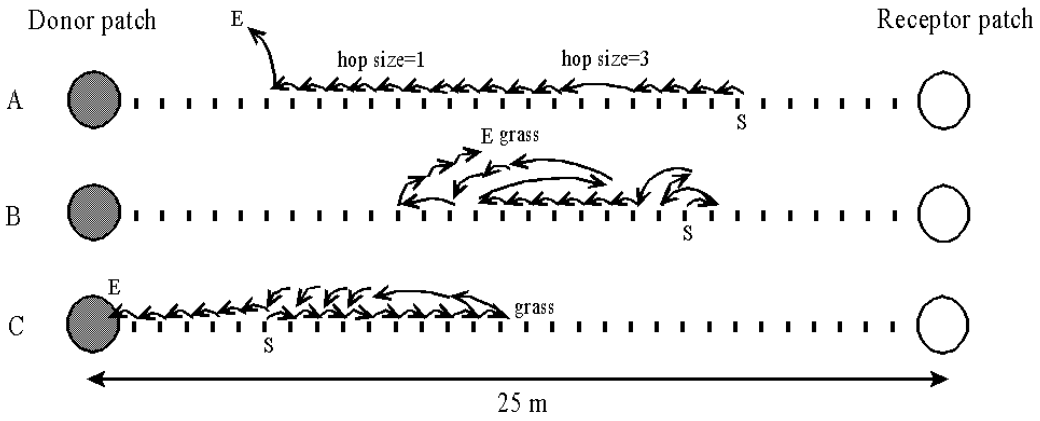

Figure 2. Examples of the behaviour of individual syrphids on a corridor. A and B. *Eristalis tenax* in a *Scabiosa columbaria* male corridor on September 18, 1997, C. *Helophilus pendulus* on a *S. columbaria* female corridor on September 11, 1997. S=start, E=end of the foraging bout.

Hop sizes were calculated for three syrphid species separately: *E. tenax*, *H. pendulus* and *E. arbustorum* visiting a *S. columbaria* female corridor where maximal guidance effect but also maximal pollen loss was expected, and an *Aster* corridor with an unknown guidance effect but with a pollen loss in the corridor and a possible heterospecific pollen deposition in the receptor patch. Syrphid species did not differ in hop size if the corridor consisted of *S. columbaria* female heads (Figure 3, Oneway Anova $P>0.05$). In the *Aster* corridor all three syrphid species made larger hops than on the *Scabiosa* corridor. On the *Aster* corridor hop size of *E. arbustorum* was significantly higher than hop size of the other two insect species (Mann-Whitney U test, $P<0.005$). With respect to the pollination of the plant (lumping the data of all insects), no difference in hop size was found between hop size on a corridor with female or male *S. columbaria* heads (Figure 4, SNK-test, $P>0.05$) but hop size in the *Aster* corridor was significantly larger than in the other two corridors (SNK-test $P<0.05$). Making large hops on an *Aster* corridor (compared with *Scabiosa* corridors) may result in a quick arrival in the receptor patch, with probably a smaller loss of *S. columbaria* pollen in the *Aster* corridor compared to a conspecific corridor. However, in the presence of an *Aster* corridor, deposition of *Aster* pollen in the receptor patch of *S. columbaria* is possible.

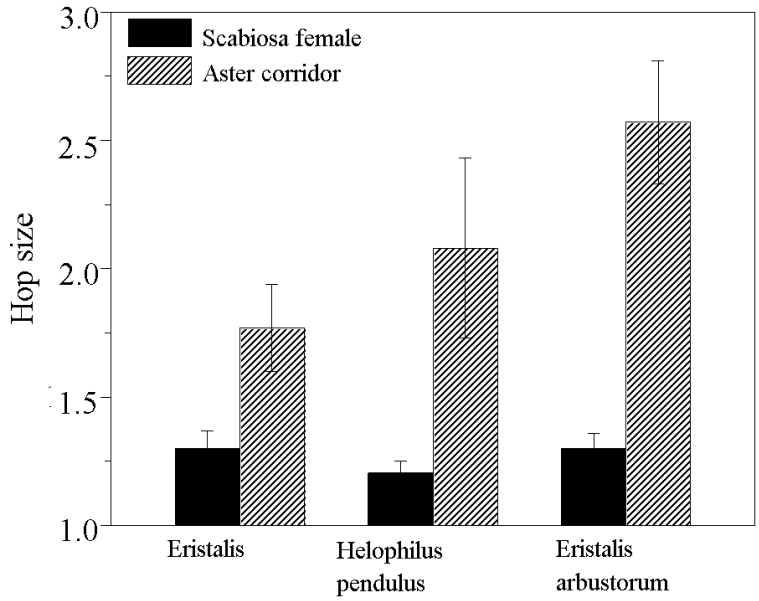

Figure 3. Hop size (mean ± s.e.) of the three most important insect species in corridors of different plant species (observations of 4 dates lumped, see Table 1). Values differed significantly if indicated by different letters (SNK test, $P<0.05$). Insect species did not differ in hop size in a *Scabiosa columbaria* corridor.

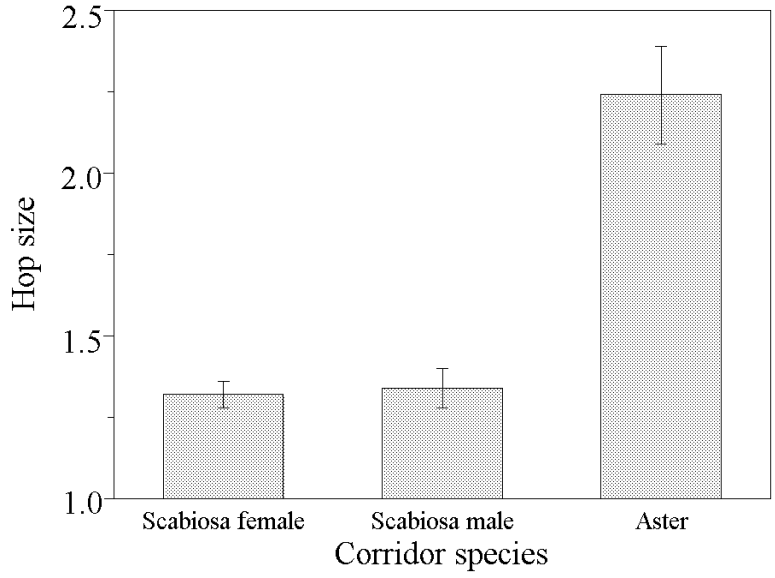

Figure 4. Hop size (mean ± s.e.) as the plant (*Scabiosa columbaria*) experienced (insect species lumped). Only hop size on the *Aster* corridor differed significantly from hop size on the other two corridors (SNK test, $P<0.05$).

Pollen deposition in corridor

Deposition of *S. columbaria* pollen in the corridor may have occurred in all experiments but it was only measured if *S. columbaria* female or *Aster* was used in the corridor. The total number of pollen grains in the *S. columbaria* female corridor equaled the number found in the receptor patches. In general, a decrease in the numbers of pollen on heads in the corridor was found from the donor patch towards the receptor patch (Figure 5). Figure 5A shows the large difference in pollen deposition between two experiments done on the same day but in different fields. Figure 5B shows that *S. columbaria* pollen was also deposited in the *Aster* corridor but the numbers were smaller and the decrease with distance to the donor patch was sharper than in *S. columbaria* female corridors.

Pollen deposition in receptor patches

Depending on the plant species in the corridor, we expected an effect on the guidance of insects, a loss of *S. columbaria* pollen in the corridor and/or heterospecific pollen deposition. Pollen deposition in the receptor patches is expressed as the ratio of deposition in the receptor patch connected with a corridor and the receptor patch not connected with the donor patch, the control receptor patch (Figure 6A). A ratio higher than 1 indicates that the presence of a corridor had a positive effect on the number of *S. columbaria* pollen deposited in the connected receptor patch. In those cases where *S. columbaria* was the corridor species, the presence of a corridor increased the pollen flow significantly towards the receptor patch in 4 out of 10 experiments. In only one case (a female corridor) we observed a significant negative effect. In the experiments with *Aster* or *Origanum* as corridor species only once a significant positive effect on pollen deposition was found. In 3 out of 12 experiments the presence of an *Aster* or *Origanum* corridor resulted in a lower pollen deposition. Overall, pollen deposition tended to be lower compared to the control patch, in patches connected with the donor patch with a heterospecific corridor (Figure 6A).

Four experiments with heads arranged in clusters were done (see Table 1). The data were insufficient to allow reliable conclusions on the effect of clustering of heads or inflorescences within a corridor due to the fact that also three plant species as corridor species were used. Only three experiments were done with a corridor situated in an across position instead of a parallel position. In all three experiments pollen deposition was decreased, with one experiment showing a significant decrease (Figure 6A).

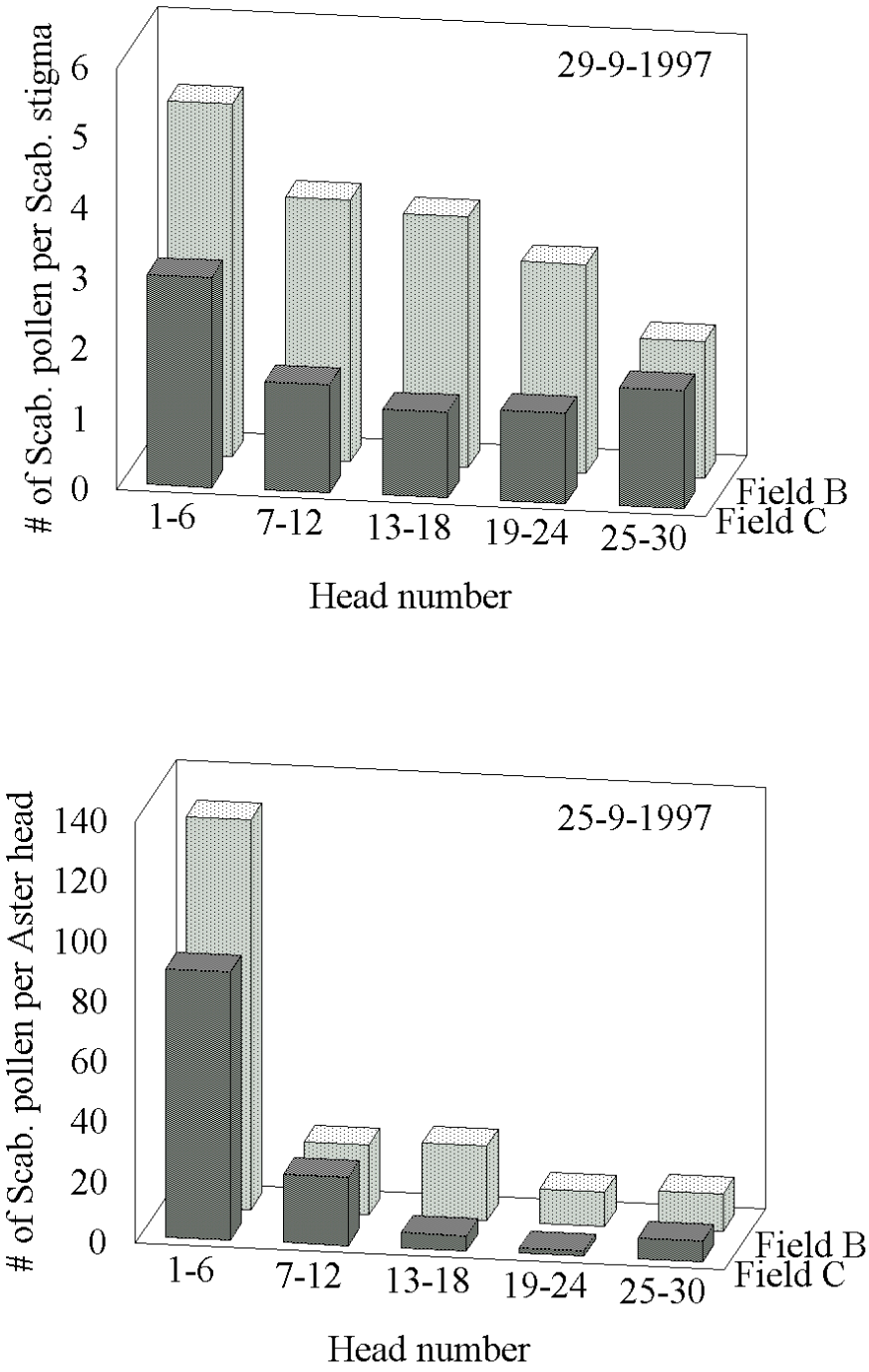

Figure 5. A. Deposition of *Scabiosa columbaria* pollen number of pollen per stigma per head (mean ± s.e.) in a *S. columbaria* female corridor in two experiments done on September 29, 1997. Head number 1-6 are close to the donor patch, 25-30 most distant from donor patch. B. Deposition of *S. columbaria* pollen in an *Aster* corridor on September 25, 1997.

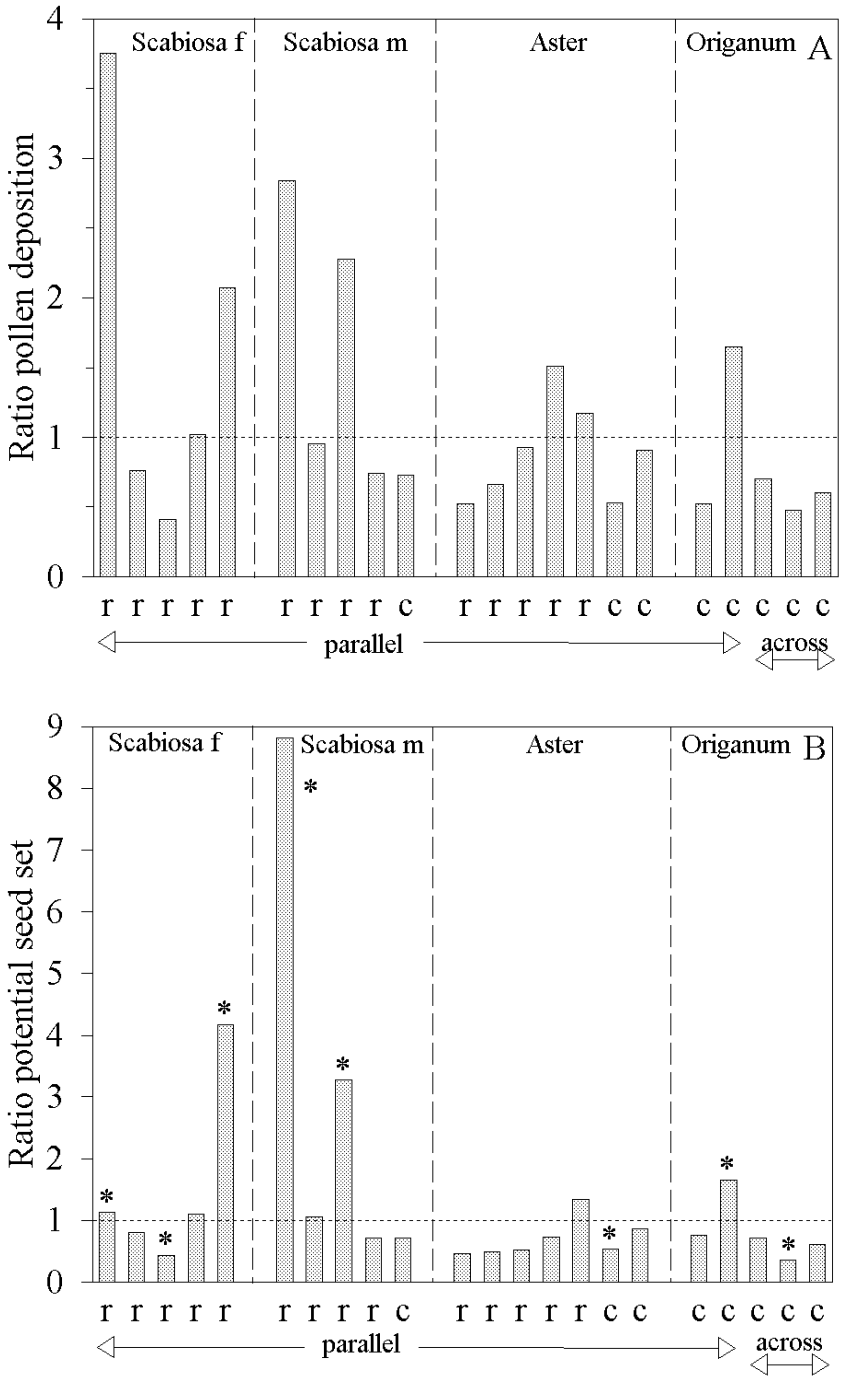

Figure 6. A. Ratio between the number of *Scabiosa columbaria* pollen per stigma per head in receptor patches connected with a corridor between the donor and receptor patch and in control patches. r=regular, c=clustered arrangement of heads or inflorescences. Stars indicate significant differences in pollen deposition per stigma between the two patches (t-test, at least $P<0.05$). B. Ratio between the percentages of potential seed set of *S. columbaria* in connected patches and control patches. Stars indicate significant differences in pollen deposition per stigma between the two patches (t-test, at least $P<0.05$).

In an earlier experiment we found that at least 4 pollen grains per stigma were needed to achieve the maximal seed set of one seed per flower (chapter 3). Applying this threshold of at least 4 pollen grains per stigma, we calculated the ratio between potential seed set in the receptor patch connected with a corridor and the control receptor patch (Figure 6B). The same pattern was found as observed for the mean number of *S. columbaria* pollen deposited, although the ratios in pollen deposition and potential seed set could differ largely in some experiments.

Variation in the number of visiting insects and deposition of *S. columbaria* pollen was very large. Since high visitation frequencies in the donor patch were not related with high pollen deposition in the donor patches (a non-significant negative relation was found with r^2=0.28, P>0.1, Figure 7A), a relation between visitation frequency and deposition in control receptor patches, where pollen deposition was depended also on visitation in the donor patches, was not to be expected. However, pollen deposition in the donor patch was positively correlated with pollen deposition in the control receptor patch (Figure 7B, r^2=0.57, P<0.01).

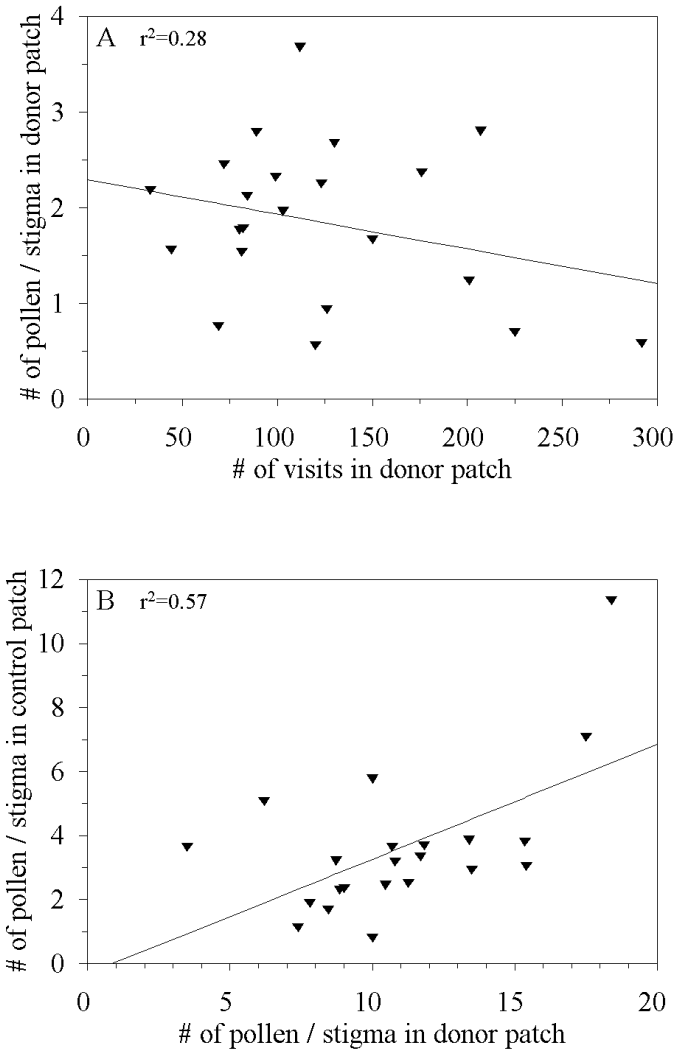

Figure 7. A. Visitation (number of visits of Apidae and larger Diptera per 60 observation surveys) and pollen deposition per stigma per hour in donor patches of *Scabiosa columbaria* (y=2.3-0.0036x, r^2=0.28, P>0.1). B. Pollen deposition (number of *S. columbaria* grains per stigma per head) in donor patches and control receptor patches of *S. columbaria* (y=-0.3+0.35x, r^2=0.57, P<0.01).

Heterospecific pollen deposition

The percentages *Aster* pollen in receptor patches were significantly higher if patches were connected with an *Aster* corridor than without corridor (Table 2). Control receptor patches received *Aster* pollen, but the numbers were much lower although still reasonable.

Table 2 Percentages *Aster* pollen (mean ± s.e.) as fraction of the total number of *Aster* and *Scabiosa columbaria* pollen per head in control patches and patches connected with an *Aster* corridor; * P<0.05, ** P<0.01, *** P<0.005.

Date (1997)	Percentage *Aster* in control patch	Percentage *Aster* in connected patch	Statistics
September 8	21.5 ± 4.0	51.2 ± 5.8	t-test 4.25 ***
	12.2 ± 3.4	39.0 ± 5.2	Mann-Whitney U 3.17 ***
September 11	23.8 ± 3.3	36.8 ± 5.2	t-test 7.32 ***
September 25	17.9 ± 2.3	43.2 ± 2.6	t-test 2.9 *
	35.3 ± 3.7	50.6 ± 3.7	t-test 2.11 *

Discussion

This study shows how insect pollinators may react on a corridor of heads or inflorescences of the same or another plant species and describes what the consequences are for pollen exchange between patches.

Visitation of the patches varied due to differences in years and dates of experiments during the season. Especially the experiments with *Origanum* as corridor species were done early in the season with a substantial amount of visits of bumblebees and butterflies. Visitation of *S. columbaria* patches and pollen deposition within the same field on the same day varied very much and in an unpredictable way. Therefore, no effect of the presence of a corridor on insect visitation could be detected. Possibly only a fraction of the visitors of the donor patch will reach the receptor patches. Other insects that did not visit the donor patches may have visited the corridor and/or the receptor patches. The last category was counted as visitors but apparently had no contribution as vector of *S. columbaria* pollen.

Differences between pollinator guilds of *S. columbaria* donor patches and *S. columbaria* corridors may be due to differences in head density. *Scabiosa* female corridors had fewer visitors than the donor patches, whereas *Scabiosa* male corridors received the same number of visitors as the donor patches (Figure 1). Differences in pollinator guilds of *Scabiosa* donor patches and *Aster* or *Origanum* corridors are much larger due to differences in both head or inflorescence density and plant species.

Insects that do not have absolute flower constancy can use a corridor consisting of another plant species. In our experiments we considered syrphids and to a lesser extent bumblebees as important visitors and pollinators. Both syrphids and bumblebees (mostly males) did switch between plant species. Little is known about the flower constancy of syrphid flies (but see Goulson & Wright 1998), but considering the composition of pollen loads on the bodies of insects visiting natural populations of *S. columbaria*, only 15-37% of the grains were conspecific (see chapter 2), their flower constancy is not high. Also bumblebees foraging in natural Dutch populations carried a low percentage conspecific pollen (0.5-10.6%, chapter 2).

In our experiments, it was clear that insect species differ in hop size (see Figure 3). Differences in hop size may influence conspecific pollen flow and heterospecific pollen deposition. Thus, in experiments with mainly *E. arbustorum* as visitor other results can be expected than in those where *E. tenax* or *H. pendulus* are the only visitors. However, such a difference could not found in our data because various species simultaneously were responsible for the pollen flow.

Only in four cases with *S. columbaria* as corridor species we found a significant increase in pollen deposition in patches connected with a corridor; a significant decrease in pollen deposition in the presence of a corridor was found once. However, the mean ratios of pollen deposition using a *S. columbaria* corridor were 1.52-1.60 for male and female *S. columbaria* corridors respectively (a positive effect) while for *Aster* and *Origanum* corridors were 0.79 and 0.89 respectively (a negative effect). The expected loss of pollen in a corridor, especially in a *S. columbaria* female corridor, does not to be important, as shown by the comparison of the data of male and female corridors). Guidance of insects by conspecific flowers in the corridor was much more important than pollen loss in the corridor. It seems that the presence of different plant species in the corridor may have a negative effect on pollen flow. Pollen deposition in the receptor patches was significantly influenced, in three occasions negative and only once positive. The presence of these corridors of another plant species may work in two ways: first, insects may be deterred by the corridor flowers and don't visit them. Secondly, flowers in the corridor may receive so much *S. columbaria* pollen that few grains are left on the insect's bodies in order to reach the other patch. Because we estimated that the pollen loss in an *Aster* or *Origanum* corridor was not greater than in a *S. columbaria* female corridor (see Figure 5 A and B) and we found that pollen deposition in the connected receptor patch was lower after an *Aster* or *Origanum* corridor, we conclude that corridors with another plant species had a deterrent effect on the behaviour of insects visiting *S. columbaria*.

No conclusion could be drawn about the effect of head or inflorescence arrangement in the corridor (in clusters or regularly distributed) on pollen flow because of shortage of data. In a similar experiment, Manasse (1992) and Cresswell (1997) could not demonstrate a net effect of clumping on gene flow, although the behaviour of pollinators changed. In two experiments, done on August 8, 1996, with a parallel corridor of *Origanum* in clusters, gene flow of *S. columbaria* pollen was measured by analysing the dispersal of allozyme marker alleles (using non-emasculated patches, data not presented in this chapter). No significant effect of the corridor on gene flow was found. The mere distance effect of 25m was much larger than the effect of the presence of the corridor. Fluorescent dye powder was also used in those experiments and despite a considerable loss within the corridor, similar amounts were deposited in connected and control patches. We did not find a relation between wind direction and/or wind speed and the ratio of pollen deposition in donor and receptor patches.

In several species, fruit and/or seed set was decreased after heterospecific pollen deposition (Galen and Gregory 1989; Kwak and Bergman 1996). Thus, visitation of a corridor containing another plant species may result in heterospecific pollen deposition, which may have a negative effect on seed set. Even if a corridor of a heterospecific plant species has a positive effect on the guidance of pollinating insects visiting the corridor species and increases pollen flow, the net impact of the corridor on seed set may still be negative. In those situations where a corridor has a negative guidance effect on insects, an additional negative effect, that of heterospecific pollen deposition, may occur. We found a reasonable percentage of *Aster* pollen (37-51%) in connected patches. The effect of *Aster* pollen on seed set was not studied.

Our study shows that the impact of a corridor on the behaviour of insects and the resulting pollen flow depends on the plant species present in patches and in corridors as well as on the pollinator species. Thus, the quality of the corridor influences the behaviour of the pollinating insects. Results of the present study are much more variable than in earlier experiments with *Phyteuma nigrum* and bumblebees as pollinators (Kwak 1994; Kwak et al. 1998; Kwak and Vervoort 2000). Behaviour of syrphids is different from that of bumblebees concerning traplining and possibly flight distances. Recapture percentage of marked bumblebees was 80-100 while the percentages of recaptures of marked syrphid individuals visiting *S. columbaria* amounted to only 4% (n=160) indicating the continuous replacement of flies.

Summarizing, the presence of a conspecific corridor has a more positive effect on pollen flow than a heterospecific corridor despite the loss of conspecific pollen in a *S. columbaria* female corridor. The visitation by insects of a heterospecific corridor results in a considerable amount of heterospecific pollen deposition. The scale of our experiments permits only conclusions on interpatch movements and not on interpopulation movements.

Acknowledgements
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Effects of pollination system and plant architecture on genetic erosion in fragmented habitats: a theoretical study

With F.J. Weissing

Summary

Fitness effects of genetic erosion were studied, with special attention to the interactions between plants and their animal pollinators. Pollinator behaviour is modeled as a 'decision rule', which determines gene flow patterns and hence the genetic structure of the plant population. Furthermore, pollinator behaviour induces adaptations of plant architecture, i.e. allocation of resources to survival, attractivity to pollinators and investment in seeds, with obvious ecological implications for population persistence. To model genetic erosion, we concentrated on the accumulation of deleterious alleles due to mutation-selection balance, and used the frequency of deleterious mutations, mean population viability and mean vigor as indicators of population performance. Selection by the pollinators, via attractivity and seed investment, resulted in a reduced mutation load in a large population, when compared to a pure viability selection model.

We considered three different pollinator decision rules, varying in their reaction to plant attractivity: highly selective Best-of-Two (BOT) strategy, non-selective Difference-of-Two (DOT) strategy and intermediate On-its-Own. After fragmentation, purging of the mutation load was more efficient with strong pollinator selection on attractivity (BOT), reducing the impact of genetic erosion. Fragmentation might affect not only population size, but also the degree of isolation between remnant subpopulations. However, spatial population structure had only marginal effects on genetic erosion. In contrast, an increase in the selfing rate after fragmentation may substantially enhance genetic erosion. If habitat fragmentation is accompanied by a change in pollinator type, especially a switch from BOT to DOT pollinators may enhance the effects of genetic erosion. A high ecological vulnerability and a low genetic vulnerability, both induced by very selective pollinators (BOT), are two sides of the same coin.

1. Introduction

Habitat fragmentation threatens the persistence of many plant populations (Soulé 1986; Weeda et al. 1990; Caughley 1994). When natural habitats are fragmented, large, continuous plant populations are often divided into several smaller and relatively isolated patches. These small, remnant populations experience an enhanced risk of extinction due to factors as demographic and environmental stochasticity. These risks are especially important for specialized plant-pollinator systems, since the combination of two vulnerable partners will certainly increase the extinction risk of the whole system.

In addition to such 'ecological' risks, fragmented plant populations may be threatened by genetic risks as well. In small and isolated populations, allele frequencies change due to random genetic drift, allowing the spread of deleterious alleles. Moreover, homozygosity is expected to increase, giving inbreeding effects a more dominant role. How rapid such 'genetic erosion' (Bijlsma et al. 1991; Van Treuren et al. 1993a) occurs and to what extent it influences population fitness is still not well understood. The mating system of a plant species will certainly be of crucial importance, since the genetic structure of a population is strongly affected by selfing and restricted gene flow (e.g. Slatkin 1987; Hartl and Clark 1989).

In animal-pollinated plants, gene flow and hence genetic population structure is largely determined by the behaviour of their pollinators. It is therefore conceivable that the (genetic) risks of habitat fragmentation are different for insect-pollinated plants, as compared to plants that do not depend on pollinators for their reproduction. In fact, pollinator choosiness introduces an additional selective force, which might efficiently reduce the genetic load of a plant population, resulting in a reduced potential for genetic erosion. If plants carrying a larger number of deleterious mutations are not attractive to pollinators, they will rarely be visited, resulting in an efficient elimination of these deleterious alleles. As a consequence, by reducing the potential for genetic erosion, the pollinators might buffer a plant population against the extinction risk by genetic processes. By means of a suite of models, we want to investigate whether and under what circumstances, the pollination system affects the mutation load and 'genetic vulnerability' of plant populations to habitat fragmentation.

Models for genetic erosion are usually based on the idea that fragmentation allows the accumulation of deleterious, recessive mutations (e.g. Lande 1995). In most of these models, deleterious mutations have negative effects on population performance due to the reduced viability of individuals carrying many of these deleterious alleles. In this paper, we extend such viability selection models to include other selection pressures, in particular pollinator-induced selection via the mating system of the plant. In addition to viability, we consider other fitness

components like attractivity to pollinators, production of rewards and investment in seed development. To this end, we assume that the genetic constitution of a plant determines its 'vigor', i.e. its potential to acquire a certain amount of resources. The lower the number of deleterious mutation a plant harbours, the more resources it has available. We assume that vigor is positively related to three fitness components, namely viability, attractivity and seed production. Obviously, viability affects the survival of the plant itself. The attractivity of a plant to pollinators determines male fitness, i.e. the relative contribution of a plant to the pollen pool, which is thus crucially dependent on the behaviour of the pollinators. Seed production is related to successful fertilization by pollinators, but also includes all kinds of maternal effects, influencing the survival of the seeds.

The behaviour of the pollinators is a crucial aspect of our model. The model is individual-based from the plant perspective, but to keep it as simple as possible, the pollinators are indirectly represented by their 'decision rule'. Mediated by the pollinators' decision rule, the investment of a plant into attractivity is translated into its contribution to pollen export and pollen import. The impact of attractivity is studied for two different scenarios. In one of the scenarios, attractivity is important for both male and female function, as is the case for many monoecious plants. In the other scenario, attractivity effects on male and female reproductive contribution are relatively independent of each other, as may be found in some dioecious plants.

This study aims at investigating the effect of the pollinator decision rule and the relation between male and female function on genetic erosion. However, it does not make much sense to compare different pollination systems directly with another, because each plant species will be adapted to its specific set of pollinators. Since there will be a trade-off between the various fitness components, the allocation of resources to these components is expected to vary with the pollination system. In other words, the relative importance of fitness components, which are related to pollen export and pollen import, is strongly dependent on the type of pollinator. Consequently, the decision rule of the main pollinator has important consequences for 'plant architecture', i.e. the amount of resources that a plant invests in its own survival compared to the amount invested in advertisment and seed production. For our study, this implies that we cannot assume that plants with very choosy pollinators have the same allocation pattern as plants with non-selective pollinators. In order to make a 'fair' comparison between pollination systems, we first derive the evolutionarily stable allocation pattern corresponding to the system under study. Afterwards, we use the adaptive allocation pattern to investigate the accumulation of deleterious mutations and the genetic consequences of habitat fragmentation. In particular, we want to know whether a switch in the prevailing pollinator, accompanying habitat fragmentation, makes a plant

more vulnerable with respect to genetic erosion.

The paper is structured as follows. As a reference system, we first consider mutation-selection equilibrium in a context of viability selection and the genetic consequences of fragmentation in this context. We then extend this model to include other fitness components, related to pollen export and pollen import. We consider several variants of the model, corresponding to different pollinator decision rules and different relations between male and female function. For each version of the model, the optimal resource allocation pattern is determined by an Evolutionarily Stable Strategy (ESS) analysis. Once the optimal plant architecture has been derived, the plant population is fragmented and the consequences of genetic erosion for plant population performance are studied. Our main focus is on the effects of pollinator decision rules and the dependence between male and female function for genetic erosion in a single small population. Additionally, we investigate the impact of pollinator-mediated gene flow between subpopulations, and of increased selfing rates after fragmentation. Finally, we study the consequences of changes in pollinator type after habitat fragmentation.

2. Deleterious mutations in small populations

One of the most plausible causes for genetic erosion is the accumulation of deleterious mutations in small, remnant populations. New, deleterious alleles are continuously created by mutation and usually disappear by selection. In large populations the selection pressures are sufficiently strong to keep the mutant alleles in a low frequency (mutation-selection balance). In smaller populations deleterious mutations are more exposed to selection, which gives the opportunity for purging of the mutation load. On the other hand, in small populations deleterious alleles become easily fixed due to random genetic drift. The net effect of these opposing forces is not always clear (Charlesworth et al. 1993; Gabriel et al. 1993; Lande 1995; Lynch et al. 1995). To analyse these processes we will study in a simulation model the effects of genetic erosion due to a large number of loci. We want to investigate to what extent the mutation load will increase after fragmentation of a large, continuous population into small and relatively isolated populations. In this section we first consider the deleterious effects of mutations on viability. Later this will be extended to other selection pressures, like attractivity and seed production, which result from the interactions between plants and pollinators.

The Haldane-Muller principle

In a large population deleterious mutations are kept at low frequencies by mutation-selection balance. At a single locus the equilibrium frequency for completely recessive deleterious alleles is $\hat{q} \approx \sqrt{\mu / s}$, where μ is the mutation rate to deleterious alleles. The selection coefficient *s* represents the reduction in fitness if an individual is homozygous for a deleterious allele. As a consequence the mean fitness (i.e. viability) of the population is given by $\overline{F} = 1 - s\,\hat{q}^2 \approx 1 - \mu$ (e.g. Hartl and Clark 1989). Interestingly, the mutation load of the population, i.e. the reduction in mean viability due to deleterious mutations, is independent of the selection coefficient *s* and depends only on the mutation rate μ. This independence of the mutation load from the selection coefficient is called the Haldane-Muller principle. For partially recessive deleterious alleles a similar principle holds. Here the equilibrium frequency is $\hat{q} \approx \mu / h\,s$, where *h* represents the degree of dominance. This results in a mean fitness (viability) of the population approximately equal to $\overline{F} \approx 1 - 2\,\mu$, which is again independent of the selection coefficients *s* and *h*.

The Haldane-Muller principle in a multilocus context

While classical theory has focused on a single locus context, the mutation load in a real population is spread over many loci. To investigate whether the Haldane-Muller principle extends to the multilocus context we used a simulation model. To this end we used a large number of loci (say *n*=100), which are unlinked. Each locus has a wildtype allele with maximal fitness and a number of deleterious alleles, which all reduce fitness to a certain extent. Mutations of the wildtype to deleterious alleles and backmutations to the wildtype allele occur with mutation rate μ, which was assumed to be equal for all loci. For simplicity we assume that all mutations are completely recessive, that means that loci have maximal fitness when deleterious alleles are in heterozygous condition and that fitness is reduced by an amount *s* when they are homozygous for deleterious alleles. The value of the selection coefficient *s* was taken the same for all loci. Therefore the fitness of an individual depends on H_d, the number of loci for which it carries homozygous deleterious alleles. It is often assumed that loci interact multiplicatively (e.g. Lande 1995; Charlesworth and Charlesworth 1998; Lynch and Walsh 1998) and that individual fitness is given by $(1 - s)^{H_d}$. In contrast, we make the simplifying assumption of an additive interaction between loci, leading to fitness $F=1- H_d \cdot s$. (Since for the parameters considered have $(1 - s)^{H_d} \approx 1 - H_d \cdot s$, the differences between these two types of models are of marginal importance.)

With additive interactions between unlinked loci, one might expect that the mean fitness of the population in mutation-selection equilibrium for completely recessive alleles were $\overline{F} \approx 1 - \mu n$. In other words the mean viability of the population should only depend on the genome-wide mutation rate (2μ*n*), and be independent of the selection coefficient *s*. To investigate whether the mutation load in a multilocus context conforms to this prediction we ran a number of computer simulations. We kept the genomic mutation rate at a value of 2μ*n*=1 and varied the number of loci (*n*), the mutation rate per allele per locus (μ) and the selection coefficient (*s*). Figure 1 shows the results for six different parameter combinations. The first half of each panel (-1000 < t < 0) shows that the mean fitness of the population (mean viability, $\overline{F}$) and the mean number of loci homozygous for deleterious alleles ($\overline{H}_d$) rapidly converge to an equilibrium value. The number loci which are homozygous for deleterious alleles clearly depends on the strength of selection *s*, with lower values of $\overline{H}_d$ for higher values of *s* (compare panels A to C). This is not unexpected since, according to the mutant allele frequency $\hat{q} \approx \sqrt{\mu / s}$, the per locus homozygosity of deleterious alleles is inversely related to *s*. In contrast, the mean fitness of the population, or more precisely its mean viability, is less dependent on the selection coefficient. In fact, with the product μ*n* kept constant, the actual mutation rate μ, the number of loci *n* and the selection coefficient *s* seem to be of marginal importance for the mean population fitness (compare panels 1 and 2). In this sense the results are in line with the Haldane-Muller principle. However, the mean viability is $\overline{F} \approx 0.65$ and hence larger than the predicted value 1-μ*n* = 0.5, which corresponds to a lower equilibrium frequency of deleterious alleles (not shown). The most probable explanation for this discrepancy is that the population is large (N=1024), but not infinite, giving rise to stochastic effects. In addition, it is conceivable that, even in the absence of linkage, associations between loci develop (e.g. Hartl and Clark 1989; Charlesworth et al. 1992; Charlesworth et al. 1993), violating the independence assumption on which the naive expectation $\overline{F} = 1 - \mu n$ is based.

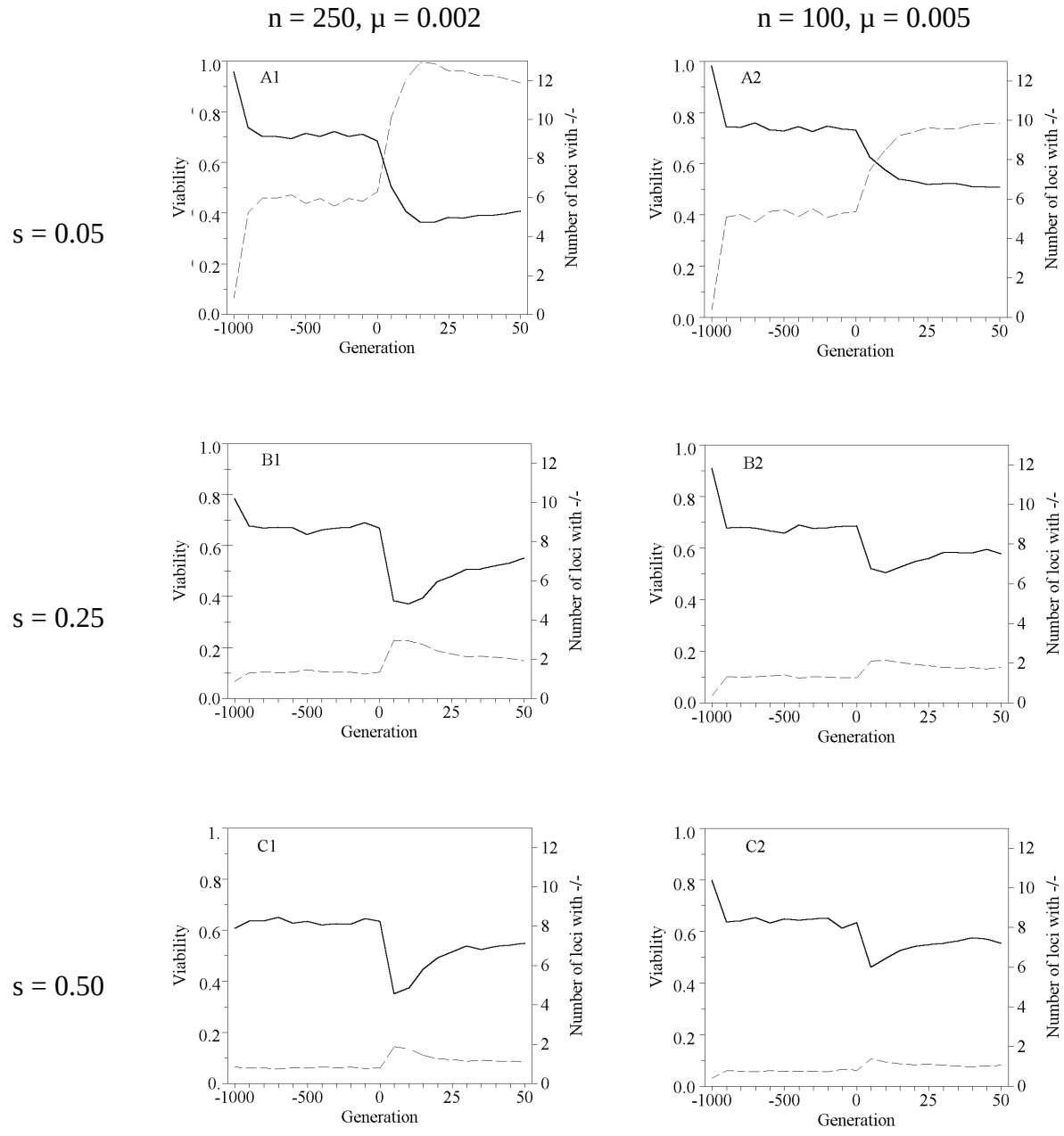

Figure 1. Mean fitness (bold) and mean number of loci with homozygous deleterious alleles (dashed) before (-1000 < t < 0) and after (0 < t < 50) fragmentation. In the first 1000 generations a single large population (N=1024) evolved to mutation-selection equilibrium. At t=0 this large population was fragmented into 64 small, completely isolated populations of N=16 individuals each. In all cases the genomic mutation rate $2\mu n$ was equal to 1. Panels differ in the values of μ and *n* (panels 1 and 2). The selection coefficient *s* is increased from 0.05 to 0.5 (panels A to C).

Fragmentation

Figure 1 also illustrates the fate of a (relatively) large population in mutation-selection equilibrium that is fragmented into multiple small and completely isolated subpopulations (64 populations with N=16 each). The effects of fragmentation on the mean fitness of the population and on the accumulation of loci with homozygous deleterious alleles are shown in the second half of the panels ($0 < t < 50$). The severe reduction in population size results in a decline in mean viability of the population within a few generations. When many loci (n=250) are involved, the number of loci with homozygous deleterious alleles doubles after fragmentation and mean fitness is reduced to half the original value. With a lower number of loci (n=100) the effects of fragmentation are less severe and the reduction in mean fitness is only half as large compared to 250 loci, even when the genomic mutation rate is equal. The selection coefficient s had only a minor effect on the decline in fitness. Although the number of loci with homozygous deleterious alleles was much higher for low values of s, this low selective disadvantage resulted in a more or less similar reduction in fitness compared to high values of s.

After a few generations purging of the mutation load starts, due to selection against homozygous deleterious mutations. The frequency of loci which carry homozygous deleterious alleles declines and mean viability rises again (e.g. Charlesworth et al. 1993; Lynch et al. 1995). This decrease in mutation load and increase in mean fitness are slightly slower when a smaller number of loci are involved. In our simulations the original mean fitness was never fully restored nor exceeded. Especially with a relatively low number of loci the mean fitness at generation t=50 remained below the original fitness of the large population, probably because we prevented fixation of alleles in the small subpopulations by allowing continuous mutation and backmutation. With a high selection coefficient s the increase in fitness due to purging of the mutation load started earlier ($t<10$ if $s \geq 0.25$, but $t>20$ for s=0.05). Strong selection also induced more rapid purging, resulting in a much shorter time with very low population fitness (less than 50 generations for $s \geq 0.25$).

To summarize, the effects of fragmentation on population fitness are most severe and the time needed for subsequent recovery is longest in case of many loci with a low mutation rate and weak selection against individuals homozygous for deleterious alleles. Although this may be the most realistic scenario for natural populations, we will for the rest of this paper focus on the scenario n=100, μ=0.005 and s=0.25. In particular, we chose a relatively small number of loci, due to computational and time limitations. But we want to stress beforehand, that in view of Figure 1 our results might be too optimistic, concerning the impact of population fragmentation in natural systems.

3. Plant-pollinator interactions and their consequences

Most studies on the effects of genetic erosion were only concerned with viability selection. In a plant-pollinator system additional selection pressures arise, which might have important implications for the (genetic) vulnerability of the plant population. Attractivity of the plants has an important role for their pollination. Therefore a relatively large investment of resources into pollinator attraction is beneficial for the plant. These high investments in attractivity can have serious negative consequences for the fitness of the plants, as is well known from the theory of sexual selection (Andersson 1994). Pollinator selectivity, i.e. a preference for more attractive plants, corresponds to a selection pressure, which is potentially stronger than viability selection. Hence pollinator selectivity contributes to the reduction of the mutation load in plant populations.

The effect of attractivity on plant fitness is mediated via the number of visits a plant receives. The number of visits is obviously related to the export and import of pollen. How attractivity differences are translated into differences in pollinator visitation strongly depends on the selectivity of the pollinator decision rule. We will here consider three different decision rules and investigate their consequences for the number of visits and accordingly pollen export and pollen import.

Pollinator decision rules

A plant's pollination depends on the number of visits it receives by the pollinators. The decision rule used by the pollinators to decide which plant to visit is therefore crucial for the distribution of visits over the plants. In our model, pollinators have a preference for more attractive plants. Plant attractivity is at least partially determined by the genotype of the plant. Attractivity has a broad interpretation, which might include the number of simultaneously opened flowers, their arrangement in inflorescences, flower size, flower colour, production of odours, etc. The plants in the population vary in their attractivity value. We distinguish three types of pollinators, which differ in selectivity, i.e. the strength of their preference for more attractive plants.

The first decision rule is based on the idea that a pollinator samples plants sequentially and that it decides for each plant separately whether the plant will be visited or not. This decision rule will be called 'On-its-Own' (OIO), since the chance to be visited, once being (randomly) sampled, only depends on the plant's own attractivity. Still, OIO results in frequency dependent selection if the total number of pollinator visits to the plant population as a whole is fixed. In that

case the actual number of visits to a given plant depends on its own probability to be visited (its own attractivity) relative to the visitation probability of the other plants (mean attractivity).

The other two decision rules are fundamentally different, since they are based on the direct comparison of different plants. The simplest of these rules is 'Best-of-Two' (BOT), where a pollinator compares two randomly sampled plants and visits the plant with the highest attractivity value. This decision rule is a special case of the more general Best-of-*n* strategy, where the best out of *n* sampled males is chosen (Janetos 1980). The Best-of-Two decision rule corresponds to highly selective pollinators, since any difference in attractivity value, however small it may be, results in large differences in the number of visits a plant will receive. We therefore included in our study a less selective, comparative decision rule which we call 'Difference-of-Two' (DOT). A pollinator applying the DOT decision rule randomly samples two plants. Its decision which plant will be visited is based on the difference in the attractivity values between the two sampled plants. If the difference in attractivity is small both plants have an almost equal probability to be visited, while a large difference in attractivity results in most pollinator visits being made to the more attractive plant. This DOT decision rule might thus represent the increase in 'errors' when a pollinator has to discriminate between plants with very similar attractivity values.

Plant visitation

Consider a population of plants, which vary in their attractivity value *A*, which varies from 0 to 1. If P(*A*) describes the distribution of attractivity values in the plant population, the mean attractivity in the population is given by $\int_0^1 P(A) \cdot A \; dA = \overline{A}$.

The plant population is visited by pollinators, which make on average $\overline{\phi}$ visits per plant. These visits are distributed over plants with different attractivity values according to the visitation function φ(*A*), which reflects the decision rule of the pollinators. Pollen export is assumed to be directly dependent on the number of visits a plant receives and thus on the attractivity value of the plant.

For decision rule OIO, pollen export is directly proportional with the attractivity of a plant: $\varphi(A) \propto A$. For decision rule BOT, the number of visits a given plant receives is proportional with the probability that the other plant sampled has a lower attractivity value:

$$\phi(A) \propto Prob(\alpha < A) = \int_0^A P(\alpha) \; d\alpha .$$

For decision rule DOT, a pollinator has to choose between two randomly sampled plants, with attractivity values A_1 and A_2, respectively. If both plants have an equal attractivity value, each has a chance of ½ to be visited. This basic probability is modified according to the difference in attractivity values, $A_1 - A_2$. For plant 1 the probability to be visited is increased if its own attractivity is higher and decreased if its own attractivity is lower than that of the other plant. The probability that plant 1 is visited is thus given by $\frac{1}{2} + \frac{1}{2}(A_1 - A_2)$. Hence, the number of visits a plant with attractivity A receives is proportional with $\frac{1}{2} + \frac{1}{2}\left(A - \overline{A}\right)$:

$\varphi(A) \propto \frac{1}{2}\left(1 + A - \overline{A}\right)$.

For all three decision rules, the constant of proportionality is easily obtained from the consistency requirement $\overline{\phi} = \int_0^A \phi(A)\, P(A)\, d\, A$.

As a result, we obtain the following relation between plant attractivity and the number of visits:

On-its-Own $$\phi(A) = \frac{A}{\overline{A}} \cdot \overline{\phi} \qquad (1a)$$

Difference-of-Two $$\phi(A) = \left(1 + A - \overline{A}\right)\overline{\phi} \qquad (1b)$$

Best-of-Two $$\phi(A) = 2 \cdot \int_0^A P(\alpha)\, d\alpha \cdot \overline{\phi} \qquad (1c)$$

All three visitation functions are depicted graphically in Figure 2.

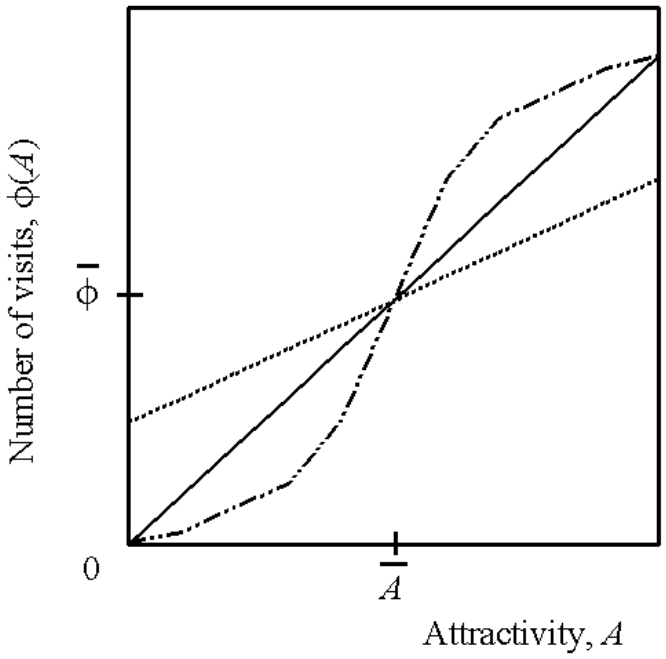

Figure 2. Relation between the number of visits a plant receives and its attractivity for different pollinator decision rules. For OIO (solid) and DOT (dotted), the number of visits is linearly related to the plant=s attractivity, while the relation for BOT (dashed-dotted) corresponds to the cumulative distribution of *A*. Notice that for OIO and DOT a plant with average attractivity receives the mean number of visits. For BOT, this is only the case when the distribution of attractivity values is symmetric, i.e. if the mean equals the median, as is assumed in this figure.

Pollen exchange and seed production

Both export of pollen to other plants and deposition of pollen on stigmas, i.e. the contribution to the next generation via male and female function, depend on pollinator visitation. Pollen export is clearly dependent on the number of visits a plant receives. In fact, we assume that pollen export and hence male function is directly proportional with the number of visits:

$$\text{pollen export} \propto \varphi(A) \qquad (2)$$

One might expect that pollen import occurs in a similar way to pollen export and is determined by the number of visits. However, several other aspects are important for the deposition of pollen. A larger nectar production, for example, generally results in a longer duration of visits (Primack and Silander 1975; Thomson and Plowright 1980; Harder 1988),

probably leading to a larger amount of imported pollen. Additionally, maternal investment in the development of seeds (e.g. endosperm production) has a positive effect on seed quality. For simplicity, we combine nectar production and other maternal effects into a single allocation component, which we call seed investment, *I*. We assume that pollen import and seed production are proportional with this seed investment and with the attraction of pollinators: seed production $\propto I \cdot \psi(A)$. The influence of pollinator attraction on pollen import, $\psi(A)$, need not be the same as on pollen export, $\varphi(A)$. For example, in dioecious plants or in plants where male and female flowering are separated in time, these two functions can be quite different. For simplicity, we consider only two extreme scenarios. In the first scenario ('type I plants'), seed production only depends on seed investment, i.e. $\psi(A)=1$. This is, for example, the case for wind-pollinated plants. In the second scenario ('type II plants'), pollen export and pollen import are governed by the same principles, i.e. $\psi(A)= \varphi(A)$. This scenario probably applies to most monoecious plants. In other words

type I: seed production $\propto I$ (3a)

type II: seed production $\propto I \cdot \varphi(A)$ (3b)

Allocation components

Viability, attractivity and seed investment are all important for the reproductive contribution of a plant to the next generation. However, plants have only a limited amount of resources to invest in these fitness components and the plant has to choose how many of its resources it will allocate to each component. We assume that a plant's capacity to acquire resources depends on its genetic constitution, that means on the number of deleterious, recessive mutations it has accumulated. This genetically determined capacity to acquire resources will be called vigor (F). The vigor of a plant is given by

$$F= 1- s \cdot H_d \quad (4)$$

where H_d is again the number of loci which carry homozygous deleterious alleles and *s* denotes the selection coefficient.

We assume that a plant has to divide its resources over three different fitness components: viability (V), affecting survival, advertisment (A), determining the number of visits, and seed investment (I), influencing pollen import and seed production. We suppose that the allocation of vigor is governed by three genetically determined allocation parameters: v for viability, a for advertisment and i for seed investment, respectively. These allocation parameters represent the relative allocation and sum to unity, $v+a+i=1$. The resulting fitness components are

$$V= v\mathrm{F},\ A= a\mathrm{F},\ I=i\mathrm{F} \qquad (5)$$

Thus the amount of resources allocated to each fitness component depends on the plant's architecture (i.e. the combination of allocation parameters) and on the genetically determined vigor of the plant.

Short-term versus long-term effects

In principle, plant vigor (F) as well as plant architecture (v, a, i) both reflect the genetic constitution of a plant. It is important to realize, however, that changes in the allocation pattern will occur on a very different time scale than changes in plant vigor. In fact, changes in plant architecture will occur on a long-term, evolutionary time scale, reflecting the adaptation of a plant to its pollinators. In contrast, changes in plant vigor may occur much more rapidly (on an ecological time scale) in response to changes in the local environment, e.g. fragmentation. For this reason we will consider changes in allocation parameters separately from changes in plant vigor.

In a first step (section 4) we investigate the expected change of v, a and i in response to pollinator behaviour. We assume that the evolution of allocation parameters takes place under favourable and relatively constant environmental conditions. Accordingly, vigor (F) is assumed to have a more or less fixed value. Given this fixed value of F we determined the evolutionarily stable allocation pattern v^*, a^* and i^*. In a second step (section 5) we investigate short-term changes in plant vigor F in response to environmental disturbance. Since on the corresponding short-term time scale the allocation pattern will hardly change, the allocation parameters will be kept fixed at their ESS values.

4. Optimal allocation patterns

Our main goal is to obtain insight into genetic erosion for various types of plant-pollinator interactions. We have to face the complication that the pollination system and plant architecture are not independent from one another. In fact, the allocation parameters of the plant will reflect the plant's interactions with its pollinators. In other words, *v*, *a* and *i* should not be viewed as arbitrary parameters, but as the result of an evolutionary adaptation process.
We use an Evolutionarily Stable Strategy (ESS) approach (Maynard Smith 1982) to determine the allocation pattern *v**, *a**, *i**, which is optimal for a given pollination system. To this end, we assume that the plant population is large and unfragmented. All plants have the same fixed vigor F. Up to a normalization factor the absolute resource investments are equal to the allocation parameters: viability $V=v$, advertisment $A=a$ and seed investment $I=i$, respectively.

ESS analysis

Quite generally, an evolutionarily stable strategy can be found as follows (Maynard Smith 1982). Consider a resident population with allocation pattern *x**, which is invaded by a rare mutant with allocation pattern *x*. The fitness of the mutant in such a resident population is denoted by W(*x*,*x**). The allocation strategy *x** is an ESS if the fitness of all possible mutants is lower than the fitness of the resident type. In other words, an ESS is given by

$$W(x^*,x^*) = \max_x W(x,x^*) \qquad (6)$$

Technically an ESS can be determined on basis of the criterion

$$\left.\frac{\partial W}{\partial x}\right|_* = 0\,,\ \left.\frac{\partial^2 W}{\partial x^2}\right|_* < 0 \qquad (7)$$

where the notation indicates that the partial derivatives are evaluated at (*x**,*x**), i.e. for *x*=*x**.

The Shaw-Mohler equation

In case of reproductive allocation, the fitness of an allocation strategy *x* in a resident population *x** is given by the Shaw-Mohler equation (Charnov 1982)

$$W(x,x^*) = \frac{1}{2}\left[\frac{m(x)}{m(x^*)} + \frac{f(x)}{f(x^*)}\right] \qquad (8)$$

In this equation *m(x)* and *f(x)* denote the reproductive contribution to the next generation via male and female function, respectively. In our case the variable *x* corresponds to an allocation pattern (*v*, *a*, *i*) which, due to the constraint *v*+*a*+*i*=1, can be represented by the two variables *v* and *a*. We assume that the reproductive contribution via pollen (male fitness) is proportional to

two factors: a plant's viability v and the expected number of visits $\varphi(a,a^*)$ that a plant with advertisment a receives in a resident population with advertisment level a^*. Male contribution is thus given by $m(v,a) = v \cdot \varphi(a,a^*)$. The reproductive contribution via seeds depends on the plant type. For plants of type I (see 3a) the production of surviving seeds (female fitness) is independent of the number of visits and depends only on viability and seed investment, $f(v,i) = v \cdot i = v(1-v-a)$. For plants of type II (see 3b) the reproductive contribution via seeds depends also on the number of visits the plant receives, $f(v,i,a) = v \cdot i \cdot \varphi(a,a^*) = v(1-v-a)\varphi(a,a^*)$. As a result we obtain two versions of the Shaw-Mohler equation, one for each plant type:

type I: $$W = \frac{1}{2} v \left[\frac{\phi(a,a^*)}{\phi(a^*,a^*)} + \frac{1-v-a}{1-v^*-a^*} \right] \tag{9a}$$

type II: $$W = \frac{1}{2} v \cdot \phi(a,a^*) \cdot \left[1 + \frac{1-v-a}{1-v^*-a^*} \right] \tag{9b}$$

ESS allocation patterns

In view of (9), the ESS values of the allocation parameters can be obtained from the criteria:

$$\left. \frac{\partial W}{\partial v} \right|_* = \frac{1}{v^*} - \frac{1}{2(1-v^*-a^*)} = 0 \tag{10}$$

and

type I: $$\left. \frac{\partial W}{\partial a} \right|_* = \frac{1}{2} \left[\frac{1}{\phi^*} \left. \frac{\partial \phi}{\partial a} \right|_* - \frac{1}{1-v^*-a^*} \right] = 0 \tag{11a}$$

type II: $$\left. \frac{\partial W}{\partial a} \right|_* = \frac{1}{\phi^*} \left. \frac{\partial \phi}{\partial a} \right|_* - \frac{1}{2(1-v^*-a^*)} = 0 \tag{11b}$$

In other words, we obtain the ESS conditions:

$$v^* = 2(1-a^*-v^*) = 2i^* \tag{12}$$

and

type I: $$\frac{1}{\phi^*} \left. \frac{\partial \phi}{\partial a} \right|_* = \frac{1}{1-v^*-a^*} = \frac{1}{i^*} \tag{13a}$$

type II: $$\frac{1}{\phi^*} \left. \frac{\partial \phi}{\partial a} \right|_* = \frac{1}{2(1-v^*-a^*)} = \frac{1}{2i^*} \tag{13b}$$

If we call $\kappa = \frac{1}{\phi^*} \frac{\partial \phi}{\partial a} \Big|_*$, we arrive at the following ESS allocation pattern:

type I: $v^* = 2/\kappa$, $i^* = 1/\kappa$, $a^* = 1 - 3/\kappa$ (14a)

type II: $v^* = 1/\kappa$, $i^* = 1/(2\kappa)$, $a^* = 1 - 3/(2\kappa)$ (14b)

or equivalently:

type I: $v^* : i^* : a^* = 2 : 1 : \kappa\text{-}3$ (15a)

type II: $v^* : i^* : a^* = 2 : 1 : 2\kappa\text{-}3$ (15b)

Dependence on pollinator decision rule

Obviously, the quantity $\kappa = \frac{1}{\phi^*} \frac{\partial \phi}{\partial a} \Big|_*$,

indicating the increase in the number of visits which a mutant with higher advertisment will receive, is of crucial importance for the optimal plant allocation pattern. In view of (1) and since $\bar{a} = a^*$. in the resident population, the expected number of visits depends in the following way on the decision rule of the pollinators (illustrated in Figure 3):

OIO: $\phi(a, a^*) = \frac{a}{a^*} \cdot \phi^*$ => $\kappa = \frac{1}{a^*}$ (16a)

DOT: $\phi(a, a^*) = (1 + a - a^*)\, \phi^*$ => $\kappa = 1$ (16b)

BOT: $\phi(a, a^*) = \begin{cases} 0 & \text{if } a < a^* \\ \phi^* & \text{if } a = a^* \\ \phi_{max} & \text{if } a > a^* \end{cases}$ => $\kappa = \infty$ (16c)

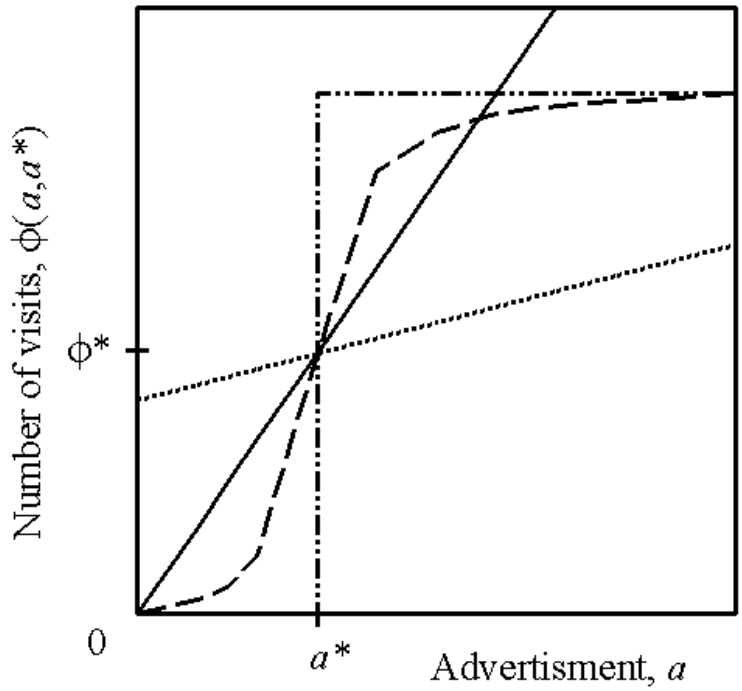

Figure 3. Expected number of visits φ(*a*,*a**) of a mutant with advertisment level *a* in a resident population with advertisment level *a** for various decision rules. In case of OIO (solid) and DOT (dotted), the number of visits increases linearly with *a*, while BOT (dashed-dotted) leads to a step-function. Less extreme choice functions will probably lead to an S-shaped function φ (*a*,*a**) which is also indicated in the figure (dashed).

Inserting (16) into (14) yields the ESS allocation pattern for all three decision rules. Notice that in case of the very selective pollinator decision rule BOT, plants should invest all their resources into advertisment, since a minute increase in advertisment results in a much higher pollinator visitation (κ=∞). Selection pressures by pollinators using the DOT decision rule are much lower. In fact the value of κ is equal to 1, which would formerly result in a negative allocation in advertisment: $a^*<0$. Of course this is impossible, but at the ESS the allocation to advertisment will be minimal ($a^*=0$), resulting in $v^*= 2/3$ and $i^*= 1/3$. Although pollinators with choice rule DOT do select plants according to their advertisment, well-adapted plants should invest no resources into this fitness component. The benefit of additional resource allocation to advertisment is in equilibrium much lower than the benefit of investing these resources into viability or seed production. For the decision rule OIO we find the following optimal allocation patterns: $\kappa = 1/a^* = 4$ for type I and $\kappa = 1/a^* = 2.5$ for type II plants (Table 1).

Table 1 ESS allocations to viability (v^*), advertisment (a^*) and seed investment (i^*). For plant type I male and female contribution are independent, whereas for plant type II female contribution depends also on the number of visits to the plant.

Decision rule	plant type	viability, v^*	seed investment, i^*	advertisment, a^*
On-its-Own	I: $f = iv$	0.5	0.25	0.25
	II: $f = \varphi iv$	0.4	0.2	0.4
Diff-of-Two		0.67	0.33	0
Best-of-Two		0	0	1

ESS allocations for a partially selfing plant

Many hermaphrodite plants are self-compatible and undergo regular selfing (Charlesworth and Charlesworth 1987a). Selfing changes the relative importance of allocation to male and female reproductive structures. For a plant with a fixed selfing rate S, the fitness contribution of selfed seeds depends only on female reproductive effort, whereas the fitness of outcrossed seeds depends on both male and female reproductive allocation. This results in a modified version of the Shaw-Mohler equation:

$$W(x,x^*) = S\,\frac{f(x)}{f(x^*)} + (1-S)\,\frac{1}{2}\left[\frac{m(x)}{m(x^*)} + \frac{f(x)}{f(x^*)}\right] \qquad (17)$$

Calculations analogous to those in the absence of selfing give the ESS allocation pattern for partially selfing plants:

type I: $v^* : i^* : a^* = 2 : 1+S : \kappa(1-S) - (3+S)$ (18a)

type II: $v^* : i^* : a^* = 2 : 1+S : 2\kappa - (3+S)$ (18b)

With help of (16) the ESS allocations can be solved for our decision rules (Table 2).

Table 2 ESS allocations to viability (v^*), advertisment (a^*) and seed investment (i^*) for partially selfing plants with fixed selfing rate S. For plant type I male and female contribution are independent, whereas for plant type II female contribution depends also on the number of visits to the plant.

decision rule	plant type	viability, v^*	seed investment, i^*	advertisment, a^*
On-its-Own	I: $f = iv$	$\frac{1}{2}$	$\frac{1+S}{4}$	$\frac{1-S}{4}$
	II: $f = \varphi iv$	$\frac{2}{5+S}$	$\frac{1+S}{5+S}$	$\frac{2}{5+S}$
Diff-of-Two		$\frac{2}{3+S}$	$\frac{1+S}{3+S}$	0
Best-of-Two		0	0	1

Simulation approach

A limitation of the ESS approach is that it assumes a monomorphic resident population, which is invaded by a single mutant. In contrast, natural populations are often highly polymorphic. We therefore ran a number of individual-based computer simulations in order to investigate the robustness of the ESS predictions (12) and (15). To this end, we consider a large population of diploid plants (N=1024), whose allocation patterns are determined by the alleles at three allocation loci. The population is potentially highly polymorphic, since at each locus a broad spectrum of alleles (with allelic values ranging from 0 to 1) is feasible. For each plant, the allocation to a given fitness component is proportional to the sum of the two allelic values at the corresponding allocation locus. Hence, for a plant with 'allocation alleles' v_1, v_2, a_1, a_2, i_1, i_2 the allocation to viability is given by $(v_1+v_2) / (v_1+v_2+a_1+a_2+i_1+i_2)$. The resulting allocation pattern determines the plant's expected viability, number of visits and seed production. Based on these expectations, the real values are determined by a chance process. In addition to selection, the population is affected by genetic drift and mutation. Mutation is modeled as follows: with probability μ (μ=0.0001) per gamete and generation, a mutation is created which differs from the original allele by a value that is drawn at random from the interval [-0.01,+0.01]. Figure 4 shows that in all cases the population converged rapidly to an allocation pattern that closely resembles the ESS predictions (indicated by arrows). This shows that the ESS predictions are quite robust. The decision rule Best-of-Two led to rapid extinction of the population, because the investment in viability evolved to zero and no seeds survived to the next generation (not shown). Although it is theoretically possible that natural selection drives a population to extinction, we consider this not very likely for real populations. We therefore assume from now on, that none of the allocation parameters can be zero. More specifically, for the rest of the paper, we assume that the relative allocations cannot drop below 5% of the total available resources.

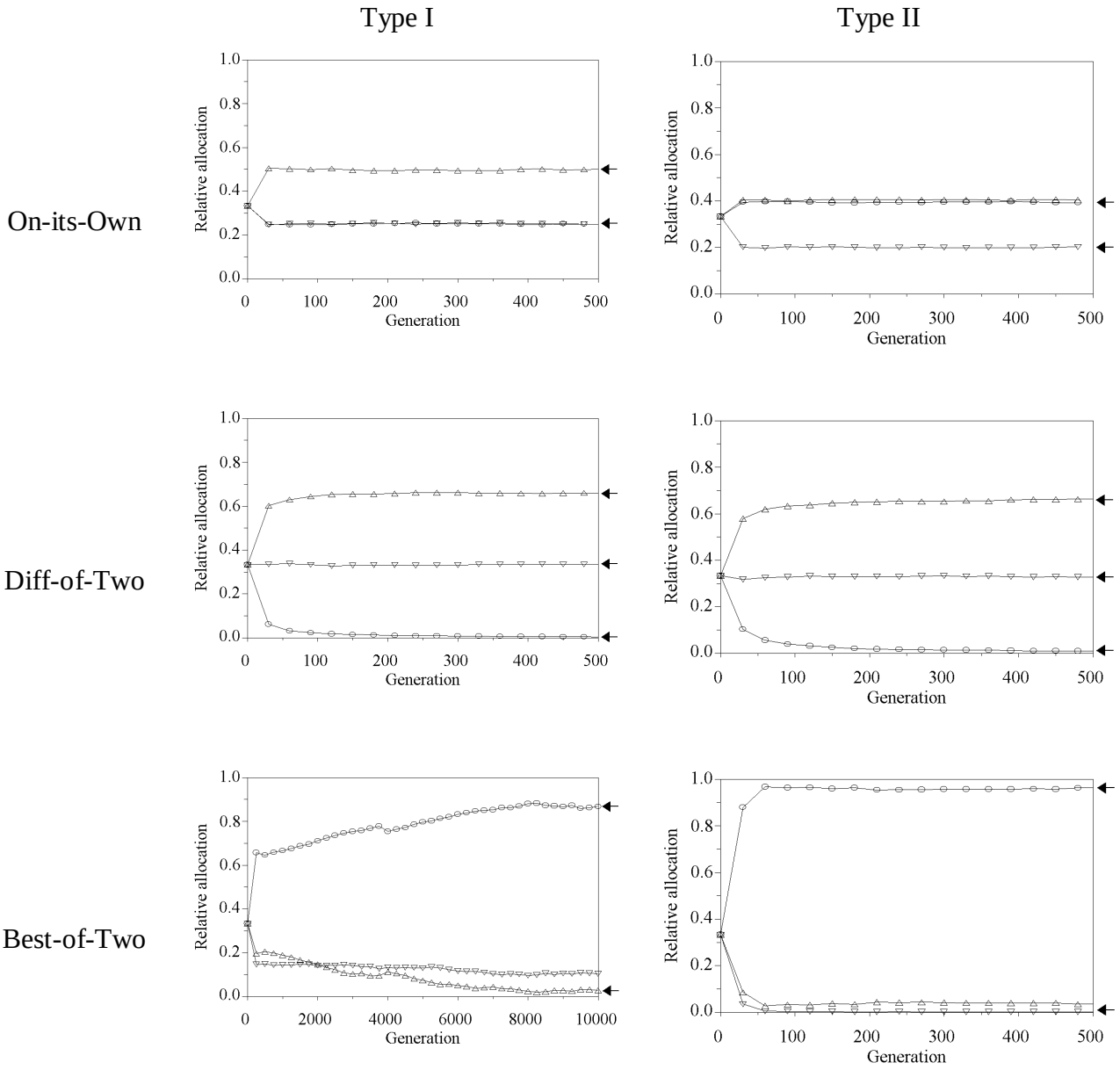

Figure 4. Comparison of ESS predictions (arrows) and the outcome of individual-based Monte Carlo simulations. For various pollination mechanisms the evolution of allocation parameters is shown for a large, unfragmented population (N=1024). Mutation rate μ was 0.0001 and the maximal mutation step size was 0.01. Means for 10 replicate simulation runs are given. Note that for BOT the time scale with plant type I ($f = iv$) is much longer than for the other simulations (panel C1). Viability: upward triangle; advertisment: open circle; seed investment: downward triangle.

Conclusions

Obviously, the pollination system is of crucial importance for plant architecture, i.e. for a plant's optimal allocation of resources to viability, advertisment and seed production. The pollinator's decision rule is more important than the plant type for the optimal allocation pattern. In fact, only for pollinators with decision rule OIO does the plant type matter at all. Not surprisingly, in this case allocation of resources to advertisment is lower in plants where seed production is independent of the number of visits (type I plants). However, the pollinator's decision rule is the dominant factor determining the ESS allocation pattern. The decision rules DOT and BOT induce relatively extreme plant allocation patterns, while decision rule OIO, which leads to intermediate selectivity (see Figure 3), induces a mixed allocation pattern. The extremely low allocation in viability, which is induced by decision rule BOT, results in an enhanced vulnerability of the plants. Notice that these specific adaptations to the pollinator can have implications for fragmentation, because habitat fragmentation can be accompanied by a shift in the main pollinator type.

5. Genetic erosion in a plant-pollinator system

Now that we have derived the optimal allocation pattern for a given pollination system, we can address the effects of habitat fragmentation on genetic erosion. From now on, we keep the allocation patterns fixed at their optimal values (with a minimal allocation of 0.05) and investigate whether and to what extent the mutation load is affected by fragmentation.

Simulation model

The simulation model is similar to the model for genetic erosion in a viability selection context (Figure 1). We now include allocation to viability V, advertisment A and seed production I. For each pollination system the allocation parameters are fixed at their ESS values, with the proviso that at least 5% of the available resources should be allocated to each fitness component. According to the genetic constitution of the plant, its resulting vigor F and the fixed allocation parameters v^*, a^* and i^* the plant allocates V, A and I (see eqn. 5) to the three fitness components. A population comprises a fixed number of N positions. For each position male and female gametes are drawn repeatedly from a pollen pool and ovule pool, until a surviving seed results. The survival probability of a newly formed seed is given by $V=v^*F=v^*(1 - s \cdot H_d)$ and hence reflects the genetic constitution of the seedling. A surviving seedling has advertisment $A=a^*F$, which results in a number of visits $\varphi(A)$, depending on the decision rule of the pollinators (see eqn. 1). The contribution of a seedling to the pollen pool is proportional to $\varphi(A)$. The

contribution to the ovule pool depends on the seedling's investment $I=i$*F and, in case of type II plants, on the number of visits received $\varphi(A)$ (see eqn. 3). As described above, plant vigor F is determined by the number of loci carrying homozygous deleterious alleles, $F=1 - s \cdot H_d$. In all cases, we use n=100 loci, a genome wide mutation rate of 1 and a selection coefficient s=0.25. As mentioned earlier, a lower mutation rate μ and weaker selection will result in more dramatic effects than the parameter combination used here.

To study the effects of genetic erosion we monitored the mean frequency of deleterious alleles, the average number of loci homozygous for these alleles ($\overline{H}_d$) and the resulting mean vigor. Moreover, we observed changes in the mean population viability. This might in reality be an important indicator of the vulnerability of a plant population. To quantify population viability we kept track of the number of trials M, which are needed to fill the N available positions, and defined population viability $\overline{V} = N / M$. Notice that $\overline{V}$ is roughly equivalent to v*F.

We start all simulation runs with a relatively large population (N=1024), which is allowed to evolve to mutation-selection equilibrium before fragmentation takes place. For all pollination systems 1000 generations were sufficient (Figure 5, $-1000 < t < 0$). After 1000 generations the population is fragmented into many small populations (64 with N=16 each). We study different degrees of isolation and self-pollination. Finally in some of the simulations fragmentation is associated with a switch of pollinator type.

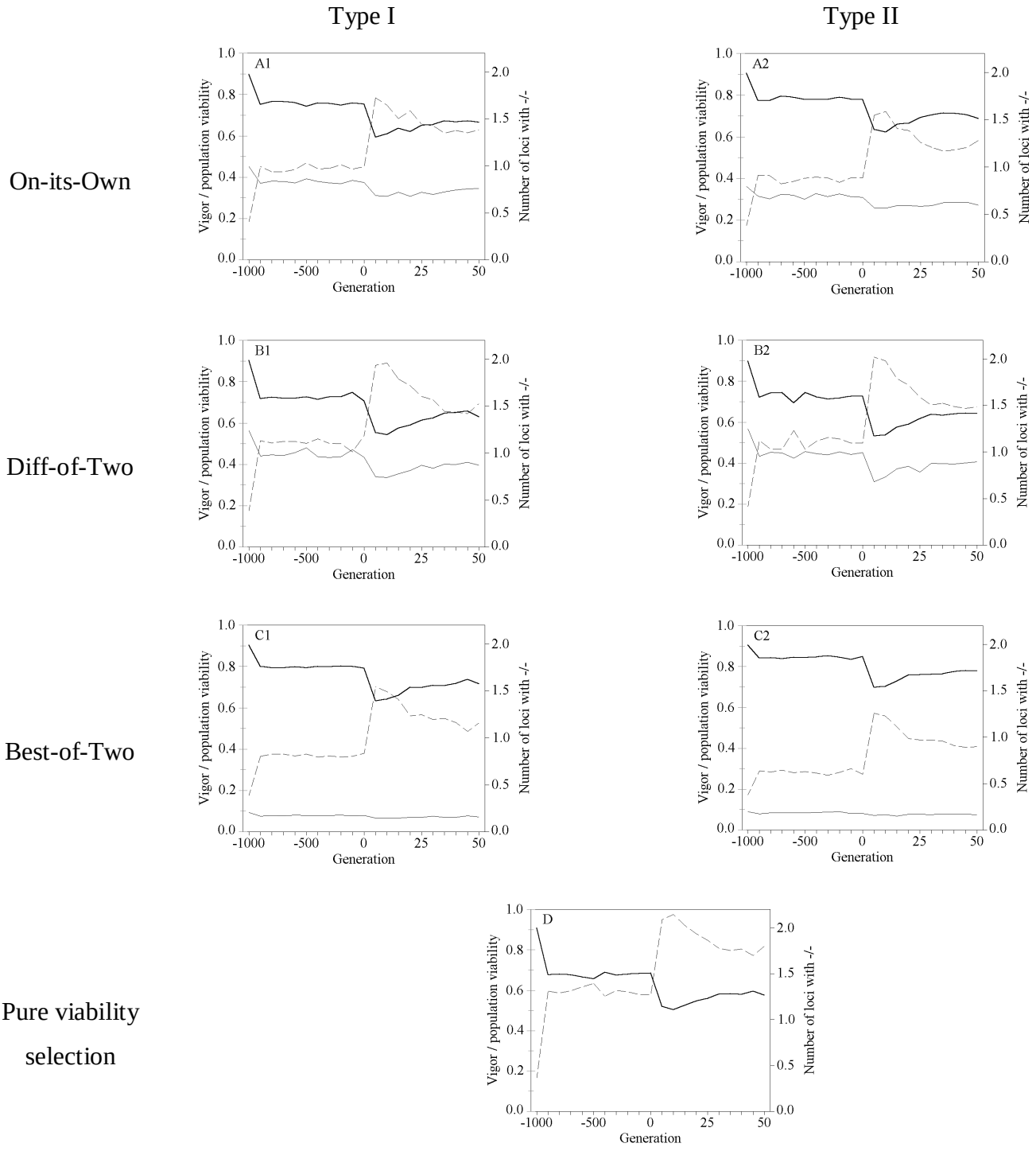

Figure 5. Effect of pollination system on the mutation load and genetic erosion. Mean vigor (bold), population viability (solid) and mean number of loci with homozygous deleterious alleles (dashed) before (-1000 < t < 0) and after (0 < t < 50) fragmentation. The effects of different pollinator decision rules are compared (panels A to C). In the first 1000 generations a single large population (N=1024) evolved to mutation-selection equilibrium. At t=0 this large population was fragmented into 64 small, completely isolated populations of N=16 individuals each. In all cases n=100 loci were used, with mutation rate μ=0.005 and selection coefficient s=0.25. Panels 1 show the results for type I plants ($f = iv$), panels 2 for type II plants ($f = \varphi iv$). Panel D (up to a change in scale the same as Figure 1 B2) gives the results for viability selection only.

Mutation-selection equilibrium

The first part (-1000 < t < 0) in the panels of Figure 5 shows how the mutation load depends on the pollination system. When compared with pure viability selection (Figure 5 D, which is equal to Figure 1 B2) the mutation load is lower when plant-pollinator interactions play a role. With respect to the number of loci with homozygous deleterious alleles, and hence also mean vigor, the most selective decision rule BOT prevented best the accumulation of deleterious mutations. It is followed by the less selective decision rules OIO and DOT and finally by pure viability selection. Accordingly, mean vigor is highest for Best-of-Two and lowest for viability selection. Interestingly, the opposite is the case for mean population viability. Since plants confronted with very selective pollinators have only few resources left to invest into viability, the mean viability of the population is highest for pure viability selection, followed by DOT, OIO and BOT. Compared to the effects of the pollinator decision rule the plant type is of minor importance. Only in case of BOT type II plants differ from type I plants: if seed production also depends on advertisment (type II) the mutation load is slightly lower and accordingly mean vigor higher.

Complete fragmentation

Figure 5 also shows the effects of population fragmentation (0<t<50). The fragments generated at t=0 were completely isolated from one another. Notice that fragmentation resulted in a rapid increase in H_d and correspondingly a rapid decrease in mean vigor. After fragmentation mean vigor drops to approximately 3/4 of the value just before fragmentation. Since population viability is roughly proportional with mean vigor ($\overline{V} \approx \nu^* \overline{F}$) the same principle applied to population viability, be it at a smaller scale. Interestingly, the drop in vigor after fragmentation was most pronounced for those systems having the lowest initial level, due to non-selective pollination (pure viability selection and pollinator decision rule Diff-of-Two). In fact, the drop in vigor for these systems was to ±73% of the original value, compared to $\geq$80% for the more selective decision rules OIO and BOT. Qualitatively, however, the dynamics of genetic erosion after fragmentation was not much affected by plant type or pollinator decision rule. In all cases, vigor gradually increased again after the initial drop, due to purging of the mutation load. However, in none of the cases, 50 generations were enough to approach the value found before fragmentation.

Partial isolation between subpopulations

Obviously, the genetic consequences of fragmentation are most drastic in case of complete isolation. In natural systems, isolation will often be less complete than simulated in Figure 5. To simulate incomplete isolation, we modified our simulation model by allowing a certain fraction of between-population pollen flow. In the modified model female gametes are always drawn from the local ovule pool, while male gametes are drawn with probability λ from the local pollen pool and with probability 1-λ from the pollen pool of another small population. Hence, λ is a measure for the degree of isolation, λ=1.0 corresponding to complete isolation (as in the simulations of Figure 5). Concerning between-population pollen flow, we consider three scenarios for the choice of the pollen donor: 'random', 'nearest neighbour' and 'attractivity-based'. For scenario 'random', the pollen donor is picked from the pollen pool of a randomly chosen subpopulation. With 'nearest neighbour', a linear arrangement of subpopulations is assumed and a foreign pollen donor is picked from one of the two adjacent subpopulations. The scenario 'attractivity-based' reflects the idea that the cumulative advertisment of all plants in a subpopulation determines the attractivity of the subpopulation to pollinators. In this scenario, the chance of a given subpopulation to contain a pollen donor depends on the mean advertisment of the plants in that subpopulation.

As illustrated by Figure 6 the three scenarios hardly differed in their effects on genetic erosion. For a given degree of isolation (Figure 6A), the dynamics of genetic erosion was qualitatively similar to that in case of complete isolation, but, not surprisingly, less pronounced for smaller values of λ. Figure 6B shows the mean vigor after 10 generations of fragmentation for the three scenarios and various values of the isolation parameter λ. Mean vigor $\overline{F}$ at t=10 decreased slowly from 96% of the original value for λ=0 to 77% for λ=1. This reduction in vigor occurred gradually with increasing isolation. Notice that for λ<0.6 mean vigor is hardly affected by fragmentation, but even for larger values of λ the effects of fragmentation on vigor are limited.

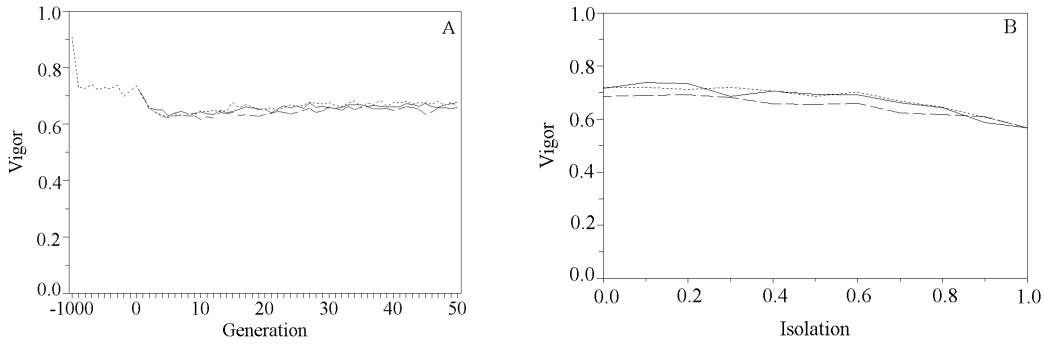

Figure 6. Effect of different scenarios for the choice of the pollen donor with incomplete isolation between population fragments: 'random' (solid), 'nearest neighbour' (dashed) and 'attractivity-based' (dotted). (A) Changes in mean vigor over time with λ=0.8. (B) Relation between mean vigor after 10 generations of fragmentation and the degree of isolation, λ. Simulations were performed with plant type II ($f = \varphi iv$) and pollinator decision rule Diff-of-Two. A large population (N=1024) was fragmented into 64 subpopulations (N=16 each) and we used μ=0.005, n=100, s=0.25.

Increased selfing

Thusfar, we have focused on the effects of fragmentation per se, without considering the possibility that fragmentation is associated with changes in the pollination system. For example, it is conceivable that the lower availability of plants for pollinators in small populations leads to an increase in the selfing rate. Such an increase in selfing will have a twofold effect. On the one hand, increased selfing will increase mean homozygosity, leading to a more pronounced drop in plant vigor and a more efficient purging of the mutation load. On the other hand, the plants are no longer optimally adapted to their pollinators, since the optimal allocation pattern critically depends on the selfing rate (see Table 2).

To study these combined effects we repeat our previous simulations, but now increase the selfing rate from S=0 before fragmentation (-1000<t<0) to S=0.2 and S=0.5 after fragmentation (0<t<50), respectively. Now, the drop in vigor, which was to 75% of the original value in the absence of selfing (Figure 7A), was more pronounced (63% resp. 51% of the original value, Figure 7B and 7C), when the selfing rate was increased. However, this drop in vigor was transient. Since the increased level of homozygosity, associated with selfing, leads to an enhanced exposure of deleterious alleles to selection, these deleterious mutations are relative efficiently purged from the population. As a result, after about 50 generations mean vigor reached almost the same level, independent of the selfing rate.

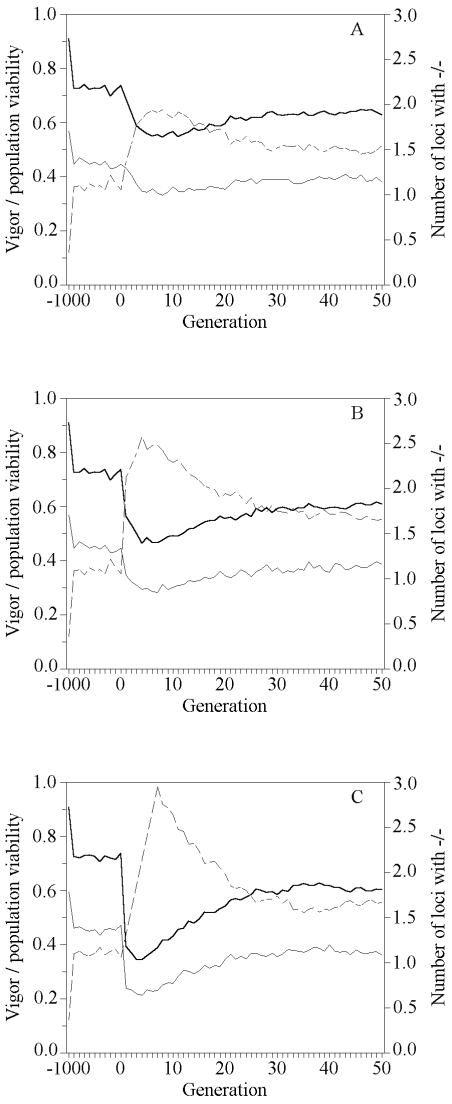

Figure 7. The effects of an increase in the selfing rate after fragmentation on plant vigor (bold), population viability (solid) and the number of loci with homozygous deleterious alleles (dashed). A large, non-selfing population (N=1024) was fragmented into 64 small (N=16 each), completely isolated subpopulations (*S*=0, panel A). The other panels show the results when, at the moment of fragmentation, also the selfing rate was increased from *S*=0 to *S*=0.2 (B) or *S*=0.5 (C) respectively. We used type II plants ($f = \varphi iv$) with decision rule DOT and μ=0.005, *n*=100, *s*=0.25.

Change in pollinator type

Another factor, that will frequently be associated with fragmentation, is a change in the abundance of pollinator species. It is easily conceivable, that the original pollinator of a plant species is replaced by a new one. This might have implications for genetic erosion, since the plant's allocation pattern will no longer be optimally adapted. To investigate the effects of a change in pollinator type we run the simulations anew, but switching the pollinator type at the moment of fragmentation (t=0). The results are illustrated in Figure 8.

In general, a switch in pollinator type had a relatively minor effect on the drop of vigor after fragmentation (although the drop was somewhat higher, when the switch was to the least selective pollinator, using decision rule DOT). However, the change in pollinator type had a marked effect on purging, that means the recovery of vigor after fragmentation. Generally, recovery was fastest for original pollinators of low selectivity and new pollinators of high selectivity. In fact, the most efficient purging occurred for a switch from DOT to BOT (Figure 8 B3), while vigor remained at a low value, when pollinator type BOT was replaced by DOT (Figure 8 C2). Obviously, the population viability was mainly affected by the allocation pattern, which, in turn, was adapted to the original pollinator type.

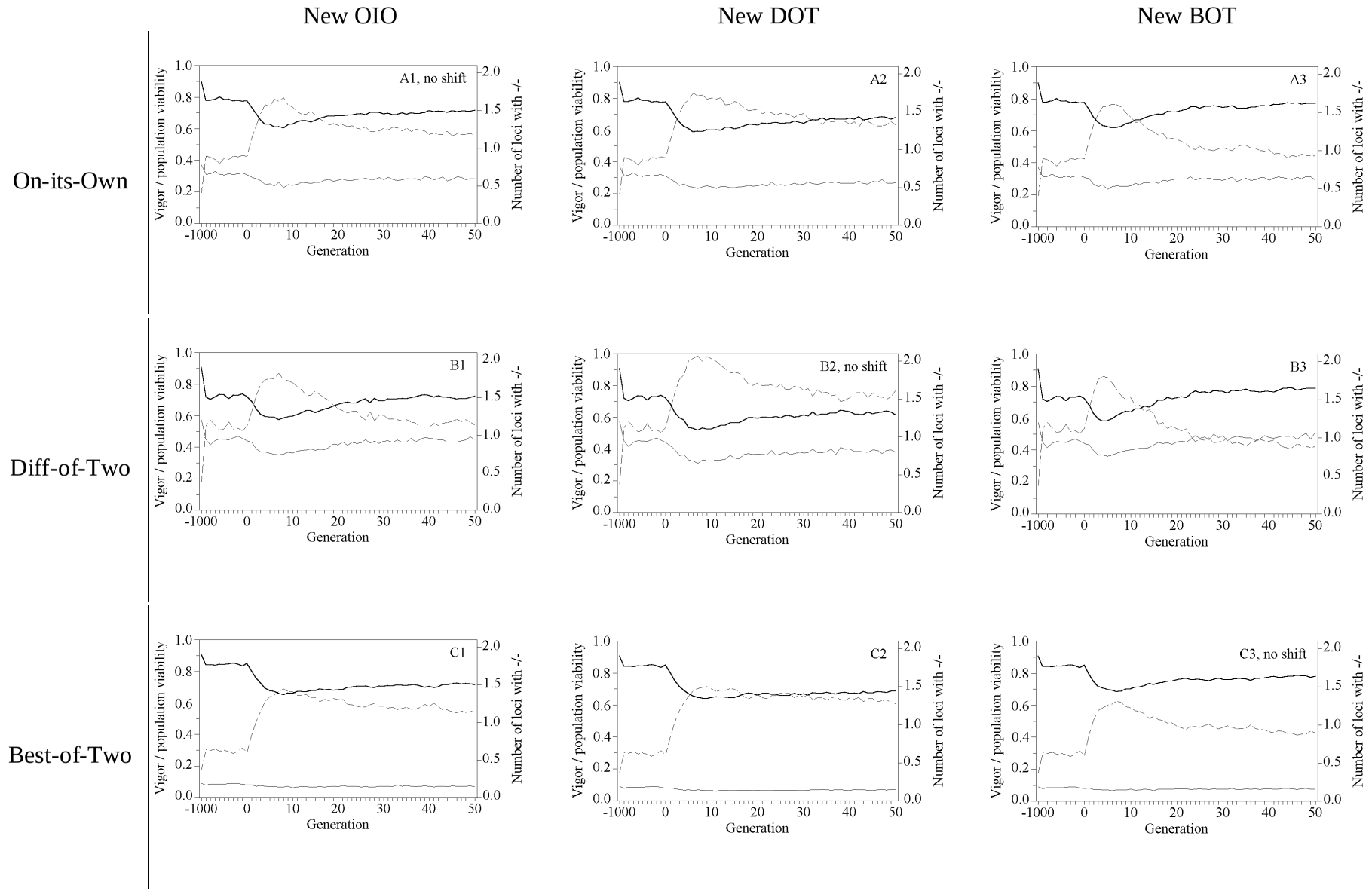

Figure 8. Effects of a switch in pollinator type after fragmentation on plant vigor (bold), population viability (solid) and the number of loci with homozygous deleterious alleles (dashed). A large population (N=1024) was fragmented into 64 small (N=16 each), completely isolated populations. At the moment of fragmentation also the pollinator decision rule was changed. Results are shown for all possible switches in decision rules (A, B, C: same original pollinator type; 1, 2, 3: same new pollinator type). We used plant type II ($f = \varphi iv$) and μ=0.005, n=100, s=0.25.

6. Discussion

Genetic erosion and population persistence

With no doubt, ecological risks are of major importance for the persistence of plant-pollinator systems. In this paper, we did not address these ecological risks, but focused on the role of genetic erosion, which may also influence the extinction risk of animal-pollinated plants. The exact relation between genetic erosion and the probability of population extinction is still unknown. Therefore, we did not model the ecological consequences of genetic erosion explicitly. In fact, we kept population size constant, making extinction of the plant population impossible. We also neglected the possibility that habitat fragmentation may result in lower pollinator visitation rates and insufficient pollination. In natural populations, a reduction in vigor will often lead to a reduced seed production and a decline in population size. Pollen limitation of seed set is another plausible consequence of habitat fragmentation, although the degree to which plants suffer reduced seed set might vary (e.g. Byers 1995; Ramsey 1995; Le Corff et al. 1998; McCauley and Brock 1998; Totland et al. 1998). If we had allowed varying population sizes and a reduction in pollinator visitation after fragmentation, many of our populations would probably have gone extinct due to pollinator limitation. Instead, we used the frequency of deleterious mutations, mean population viability and mean vigor as indicators of population performance. However, at the moment the 'ecological' relevance of these indicators is unclear.

Fitness consequences of genetic erosion

To model genetic erosion, we concentrated on the accumulation of deleterious alleles due to mutation-selection balance. The genomic mutation rate, that means the total number of mutations per individual per generation, is of crucial importance. Estimates of the genomic mutation rate usually vary between 0.2 and 2.0 mutations per individual per generation (Lynch and Walsh 1998), but see Crow (1999). Here, we used a genomic mutation rate of U=1.0. In theory (see section 2) only the *genomic* mutation rate appears to matter for genetic erosion, rather than the per locus mutation rate μ. This allowed us to compensate for a relatively low number of loci (n=100, speeding up the simulations) by a relatively high mutation rate per locus. However, the theoretical expectation that only μn matters, which was derived for infinite populations, seems to hold only approximately for the smaller populations considered here. In fact, for a given genomic mutation rate, the effects of genetic erosion seem to be more severe in case of a larger number of loci with a smaller mutation rate per locus (see Figure 1). By using a relatively small number of loci, we will thus have underestimated the consequences of genetic erosion in our simulations.

According to the Haldane-Muller principle (see section 2), the effect size of deleterious mutations (the selection coefficient *s*) does not affect genetic erosion, at least in an infinite population. Again, in the finite populations studied here, the Haldane-Muller principle only holds by a rough approximation. It was found that genetic erosion had the strongest effects for the smallest values of the selection coefficient *s* (see Figure 1). Empirical estimates of the average effect size of deleterious mutations vary enormously, but they are usually considerably smaller than the selection coefficients chosen in our simulations (s=0.25) (Lynch and Walsh 1998). Hence, our simulations will probably underestimate the amount of genetic erosion.

To keep the genetic assumptions as simple as possible, we only considered recessive deleterious alleles. In natural populations, deleterious mutations will often be only partially recessive, but estimates of the fitness depression of heterozygotes, 1-*hs*, vary considerably. Our choice of *h* equal to zero will probably overestimate the effects of genetic erosion, since a small value of *h* has similar effects as a small selection coefficient *s* (e.g. Charlesworth et al. 1993). We did not study the possibility of overdominance in fitness, which might have a large impact on genetic erosion, but is generally considered a less important factor (Lynch 1991; Fu and Ritland 1994). However, even if the majority of loci correspond to the pattern of partial dominance, a small number of loci with overdominance may have a large effect, because genetic erosion due to overdominance cannot be purged.

In this paper, we kept the genetic assumptions as simple as possible, in order to focus on the fitness effects of genetic erosion that may be typical of plant-pollinator interactions. In particular, we addressed the extent to which it matters whether selection is only acting on viability (as is usually assumed in models of genetic erosion) or also on other fitness components. In our models, where also attractivity and seed investment played a role, the mutation load in a large population was reduced, when compared to a pure viability selection model (see Figure 5). Qualitatively, the system reacted in a similar way to habitat fragmentation as in the case of pure viability selection. Quantitatively, however, purging of the mutation load was more efficient with strong selection on attractivity, reducing the impact of genetic erosion.

Modeling pollinator behaviour

Pollinator behaviour (i.e. 'decision rule') is a central aspect of our model. On the one hand, the pollinator behaviour determines gene flow patterns and hence the genetic structure of the plant population. On the other hand, the pollinator behaviour has more long-lasting effects by inducing adaptations of plant architecture, with obvious ecological implications for population persistence.

To keep the model as simple as possible, individual pollinators were not modeled explicitly, but instead represented by their 'decision rule'. Although pollinator behaviour is certainly much more complex, preferential visitation of certain flowers and plants is an important aspect of their foraging strategy. It is often found that plants with large floral displays (large flowers or inflorescences with many flowers) are visited more frequently than plants with a small floral display (Klinkhamer et al. 1989; Goulson et al. 1998; Le Corff et al. 1998; Krupnick et al. 1999). Soltz (1986) found that bumblebees visited mainly nearby inflorescences, but made occasional visits to plants farther away. She suggested that these plants are more attractive to pollinators.

An important aspect that is obviously missing in our model, is the individual behaviour of pollinators, which might have an important impact on the local genetic structure of plant populations. Mainly for simplicity, we focused on small, unstructured patches. However, we also considered pollinator-mediated gene flow between subpopulations, which can be viewed as spatial structure on a somewhat larger geographical scale. In general, we only found marginal effects of spatial population structure on genetic erosion, unless the degree of isolation between subpopulations was high ($\lambda>0.6$, Figure 6). The exact way in which the effects of spatial structure were modeled was not important. We studied three different scenarios for the choice between subpopulations, which were inspired by empirical observations that pollinators might follow different flight paths between patches of flowering plants: more or less random, area-restricted searching or traplining along fixed routes (e.g. Soltz 1986; Herrera 1987; Stacy et al. 1996; Thomson 1996; Velterop and Kwak 1997). All three scenarios for the choice between subpopulations gave similar results (Figure 6). Certainly, to really quantify the effects of spatial structure on genetic erosion, a more extensive study is required, based on a spatially more explicit model with individual pollinators.

Implications of pollinator behaviour

The importance of plant attractivity for pollinator visitation differed between the pollinator decision rules. Differences between pollinator species were found empirically in studies to the reaction of insects on plant attractivity, which is usually measured as floral display, i.e. the number of simultaneously opened flowers. For example, bumblebees reacted more strongly to differences in floral display of *Wurmbea dioica* than did flies (Vaughton and Ramsey 1998). Although they found butterflies to be more selective too, in other studies butterflies were more concerned with searching mates than with foraging for food, presumably resulting in non-selective flower visitation (Goulson 1997; pers. observ.).

In our model, we considered three different pollinator decision rules, which are perhaps not sufficiently realistic, but on the other hand, might reflect, at least qualitatively, different pollination systems. One might speculate that our highly selective Best-of-Two (BOT) strategy represents a specialistic plant-pollinator interaction, while the non-selective Difference-of-Two (DOT) strategy corresponds to a generalist pollinator, which might be less 'critical' with respect to flower choice between and within plant species. In view of our result that genetic erosion is more severe in case of a non-selective pollinator (see Figure 5), one might therefore predict that, at least from a genetic point of view, specialized pollination systems are less vulnerable to fragmentation than plants with generalist pollinators.

In this paper, we have focused on the direct effects of fragmentation on the genetic composition of a plant population. However, habitat fragmentation will often be accompanied by changes in other factors, which are important for genetic erosion. It is conceivable that pollinators become less selective after fragmentation of a large population into small subpopulations, because fewer alternative plants are available for visitation in the vicinity. Other aspects of pollinator behaviour might also change. For example, Klinkhamer et al. (1994) found that bumblebee visitation sequences to isolated plants were longer as compared to large groups of plants, resulting in increased geitonogamy. As shown in Figure 7, such increased selfing rate after fragmentation may substantially enhance genetic erosion. As a consequence, self-compatible plant species might be genetically more vulnerable to habitat fragmentation, compared with self-incompatible plants.

Even more drastic than such changes in pollinator behaviour, are switches in the pollinator type, that might regularly accompany fragmentation of natural habitats (e.g. Olesen and Jain 1994; Allen-Wardell and al. 1998; Kearns et al. 1998). Our simulations showed that new, highly selective pollinators (BOT) were able to purge the genetic load quicker, and needed fewer generations to restore population viability to its original value (see Figure 8). One might expect that when switches in pollinator type occur, often (highly) selective pollinators will be replaced by less selective pollinators. As shown by our simulations (see Figure 8, panel C2), such a switch may enhance the effects of genetic erosion.

Effect of evolutionary history on plant vulnerability

By explicitly addressing the effect of the pollination system on plant architecture, we have stressed the importance of evolutionary history for the future prospects of a plant population. We are aware that our allocation model is rather simple and perhaps not sufficiently realistic. We assumed, for example, that the total amount of resources available had to be distributed over three non-overlapping compartments. This simplifies the analysis, and one should realize that a more mechanistic approach may lead to rather different evolutionary predictions (Pen and Weissing 1999). Our assumption that resource allocations translate linearly to the fitness components, was also made only for simplicity. More sophisticated models should include the possibility of condition-dependent allocation, where the allocation pattern depends on the amount of resources available (e.g. Klinkhamer et al. 1997). Even with these limitations, we are convinced that evolutionary considerations should not be neglected in the context of conservation genetics.

In our case, the combination of evolutionary arguments with an analysis of the short-term genetic consequences of fragmentation, illustrates the interplay between genetic and ecological vulnerability. On the one hand, the most selective pollinator leads to a reduced mutation load, resulting in less severe genetic erosion. On the other hand, the same very selective pollinators induce a low allocation of resources to viability. Consequently, plants with this type of pollinators will be more susceptible to seed failure, due to demographic and environmental stochasticity. Hence, a high ecological vulnerability and a low genetic vulnerability, both induced by very selective pollinators, are two sides of the same coin.

List of symbols

n	number of loci
q	frequency recessive deleterious allele
μ	mutation rate per allele per locus
s	selection coefficient
H_d	number of loci homozygous for deleterious mutations
h	degree of dominance
t	time in number of generations
F	vigor
V	total viability
A	attractivity (total advertisment)
I	total seed investment
v	allocation to viability
a	allocation to advertisment
i	allocation to seed production
$\varphi(A)$	number of visits a plant with attractivity A receives
κ	steepness of $\varphi(A)$ for plants with equilibrium attractivity, i.e. the increase in number of visits which a mutant with higher advertisment will receive (eqn. 13 and 14)
$\psi(A)$	pollen import by a plant with attractivity A
$W(x,x^*)$	fitness of mutant with strategy x in resident population with strategy x^*
$m(x)$	reproductive success via male function
$f(x)$	reproductive success via female function
N	number of individuals
$\bar{V}$	population viability
M	number of trials needed to obtain a surviving seed
S	selfing rate
λ	degree of isolation

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Summary

Habitat fragmentation and gene flow in animal-pollinated plants

During the last decades, many natural habitats became fragmented. The remaining areas can only sustain small populations, which are vulnerable to stochastic processes. The importance of demographic and environmental stochasticity is well known. In contrast, the impact of genetic stochasticity is less clear and crucially dependent on the amount of gene flow between the remnant populations (chapter 1).

In case of plants, gene flow can occur via seeds or pollen. As seed dispersal in many plant species is rather restricted, gene flow by pollen can have a considerable impact on the genetic composition of a plant population. Pollen can be dispersed by various vectors, like wind, water or animals. Despite this variation in dispersal mechanism, pollen dispersal distances generally follow a leptokurtic distribution, resulting in deposition of the majority of pollen grains close to the source plant and only incidental dispersal over larger distances. The exact shape of the pollen dispersal curve will depend on many ecological factors, especially when biotic vectors, like insects, are involved. Both the pollinator species present and their behaviour determine the efficiency of pollen transport and the distances over which pollen grains are exchanged. Pollinator species composition and pollinator behaviour may vary in response to changes in spatial population structure of plant species, such as size, density and degree of isolation.

The effects of habitat fragmentation on pollinator behaviour and the consequences for patterns of pollen flow in *Scabiosa columbaria* are the main focus of this study. In chapter 2, visitation of insects on *S. columbaria* is investigated in natural populations and their importance for pollination determined. Then, the performance of several estimation methods for gene flow is compared in our experimental study system (chapter 3). Using the same setup, the effects of habitat fragmentation on pollen flow between patches of *S. columbaria* are studied experimentally in chapters 4 and 5. Interactions between plant and pollinators and their consequences for genetic erosion after habitat fragmentation are investigated theoretically (chapter 6).

Pollination value of insect visitors for *Scabiosa columbaria*

We investigated the pollination value of regularly visiting insects on *S. columbaria* (chapter 2) in order to understand our experimental results and to elaborate these with respect to the consequences of habitat fragmentation for natural populations. In The Netherlands, *S. columbaria* was frequently visited by bumblebees and syrphid flies, while moths and butterflies were regular visitors. Additionally, in French populations, honeybees and the specialist bee species *Dasypoda argentata* were commonly observed. The insect groups were classified according to several foraging characteristics, like flight distance between subsequent flower visits (quality), purity of the pollen load (quality), number of heads visited per minute (quantity) and number of *S. columbaria* pollen grains deposited per unit time (quality). Bumblebees and syrphid flies were comparable in pollination value, foraging at a moderate speed and flying over intermediate distances between flower visits. Furthermore, both groups deposited a large amount of heterospecific pollen. Due to their high abundance, bumblebees and syrphid flies were very important pollinators in the Dutch populations. Butterflies were rather inefficient pollinators, foraging at low speed and carrying very small numbers of pollen grains. However, they were the only group regularly covering distances >10m between flower visits, making their visitation

potentially important for the genetic diversity of the plant populations. In France, bumblebees and syrphid flies were rarely observed. The most abundant visitors were *D. argentata* and honeybees. The pollination value of honeybees was similar to that of bumblebees and syrphid flies. *Dasypoda argentata* was a very good pollinator, scoring high on all foraging characteristics. Due to the absence of this specialist visitor in The Netherlands, the Dutch populations of *S. columbaria* depend on an assembly of less efficient pollinators for their pollination. Consequently, the quality and quantity of pollination may fluctuate with variation in insect species abundance. As each taxon contributes to another aspect of pollination, the presence of a diverse pollinator guild is very important for the pollination of *S. columbaria*.

Variation

Variation in time and space is inherent to experiments under (semi-)natural conditions. Insect abundance varies with the time of day, with weather conditions, seasonally and between years. Additional variation may originate from differences in the flowering surroundings and from individual variation between pollinators in body load and foraging pattern.

Large variation was indeed found in insect abundance between days, over the flowering season of *S. columbaria*, between years and weather conditions (chapters 2 and 5). Additional variation was observed between experimental patches, between the fields where they were located and between individual insects. As a consequence, the amount and pattern of pollen dispersal were also highly variable (see discussion of chapters 3 and 4). We repeated all experiments several times, spread over the flowering season. By combining the results, temporal variation was included to get an overall view of the pattern of pollen dispersal. Despite the limited number of replicates and the high stochasticity, a couple of clear conclusions could be reached.

Estimating pollen-mediated gene flow

Different techniques can be used to estimate gene flow by pollen. The relative performance of some of them was evaluated for a patchy population of *S. columbaria*. To investigate pollen dispersal after habitat fragmentation, a linearly arranged population of *S. columbaria*, consisting of three equally sized patches separated by 25m, was created in meadow without other flowering plants. Two replicates of this experimental population were located in different fields and were pollinated by naturally occurring insects. On five days, spread over a flowering season (1996), dispersal of pollen grains, a pollen analogue (fluorescent dye powder) and allozyme marker alleles was simultaneously observed (chapter 3). Direct observations of insect behaviour were not regularly included, because tracking of individually marked syrphid flies, which are important visitors of *S. columbaria* in The Netherlands, appeared to be very difficult over a longer time period.

Each method for the estimation of gene flow has its own advantages and limitations. Allozyme analysis is a relatively costly and time-consuming technique, both in the preparation of experiments and in the analysis of the seeds. Furthermore, the results may be confounded by selection on genotypes during fertilization, ripening and germination or accidental loss of seeds while manipulating the plants. It gives, however, the net effect on successful gene flow and therefore we used it for validation of the other methods. Fluorescent dye powder is applicable when genetic markers are not available. Different pollen sources can be distinguished by different dye colours, allowing multiple independent estimates of pollen dispersal in a single experiment. Fluorescent dye powder can be used without emasculation, thus avoiding possible

changes in insect behaviour in response to the presence and absence of pollen. Pollen grains of *S. columbaria* are large (50-70 µm), making pollen counts in emasculated patches a relatively quick and direct method to estimate pollen dispersal. At the end of an experiment, pollen counts are immediately available, allowing the rapid adjustment of the experimental design if necessary. For pollen counts in *S. columbaria*, emasculation is needed to identify the pollen source in this self-compatible plant species.

Variation between fields and days was large for all estimates of pollen flow, but when averaged over several replicates, the dispersal patterns of fluorescent dye powder and allozyme alleles were almost identical. Estimates of pollen flow, based on dispersal of pollen grains into emasculated patches, were also similar to dispersal of dye powder and allozymes (chapter 3). Although quantitative estimates of pollen flow differed, allozymes, dye powder and pollen grains all showed the same qualitative pattern of pollen dispersal. Emasculation had only quantitative effects on pollen flow, because patterns of pollen dispersal using pollen counts and fluorescent dye powder were still qualitatively similar over larger distances, up to 200m (chapter 4). Thus, each of these methods can be used in comparative studies, where the relative dispersal of pollen is investigated under different circumstances.

Pollinator behaviour and pollen flow in fragmented habitats

Habitat fragmentation results in increased distances between patches and often an increase in barriers, restricting pollinator foraging and gene flow. These aspects of habitat fragmentation were investigated separately with respect to their effects on pollinator behaviour and patterns of pollen flow. Pollen dispersal was compared to emasculated patches with varying degrees of isolation from a source patch of pollen. The effectiveness of corridors in reducing isolation and increasing pollen flow between patches was also investigated. These experiments used pollen counts. In the isolation by distance experiments, fluorescent dye powder was applied additionally. The observed differences in pollen dispersal between patches were extended to their genetic consequences using the results of chapter 3. The impact of (a change in) the pollination system on the effects of habitat fragmentation was studied theoretically.

Isolation by distance

Isolation by distance severely reduced pollen dispersal (chapter 4). Already at a distance of 25m, pollen deposition in emasculated patches was approximately one quarter of the deposition found in the source patch. Pollen grains rarely dispersed over 200m. Dispersal of fluorescent dye powder and allozyme marker alleles decreased even faster with distance than dispersal of pollen grains (chapters 3 and 4). Deposition of dye powder and allozyme alleles at 25m was only 10% of that in the source patch, thus pollen counts overestimated the absolute amount of effective gene flow by pollen. Although pollen dispersal decreased steeply with distance to the source patch, this was not completely reflected in the production of seeds. Seed set declined less severe with distance (chapter 4), presumably because four pollen grains per stigma are already sufficient for maximal seed production (chapter 3).

Barriers and corridors

A hedgerow or bushes may function as a barrier to insect foraging behaviour. To mimic the effects of such physical barriers a camouflage net was used in order to visually isolate a single patch. Despite the use of several replicates, pollen dispersal was too variable between fields, days and years to allow sound conclusions (chapter 4). On some days a tendency for reduced pollen flow to 'isolated' patches was found, while on other days pollen dispersal even increased. The camouflage net, which clearly was a visual barrier to humans, did not function as a barrier to pollen flow. Care should be taken in the interpretation of structures with respect to their effects on insect behaviour.

It is often suggested that corridors may enhance gene flow between (sub)populations. The effects on pollen flow between patches were investigated using different types of corridors, varying both the quality (flowering species) and arrangement within the corridors (chapter 5). The flowering species in the corridor influenced both insect behaviour and pollen flow. Visitation of flowers in a *S. columbaria* corridor with female heads resulted in a considerable loss of conspecific pollen in the corridor. Nevertheless, a tendency for increased pollen deposition in connected patches was found, compared to control patches, which were not connected with a corridor. Guidance effects increased the number of insects, flying along the corridor, and the resulting increase in pollen transport appeared to be more important than the loss of pollen on corridor flowers. In corridors consisting of *S. columbaria*, pollinators flew shorter distances between subsequent flower visits, compared to heterospecific corridors containing *Aster*. Despite these larger flight distances and potentially lower pollen losses, pollen flow between connected patches tended to be reduced, if the corridor contained *Aster*. Probably, insect pollinators are deterred by a heterospecific corridor. Furthermore, corridors containing heterospecific flower species resulted in a high deposition of heterospecific pollen in connected patches. No significant effects of corridor arrangement on pollen flow could be detected, at least with the limited number of replicates done.

Plant-pollinator interactions

Different pollinator species reacted differently to fragmentation of the plant population. For example, the presence of a flower corridor resulted in different flight distances between flower visits for several species of syrphid flies, influencing the dispersal of conspecific and heterospecific pollen (chapter 5). Besides differences in reaction to fragmentation between pollinator species, also the pollinator species composition may be affected by habitat fragmentation. The effects of such changes in the pollination system after habitat fragmentation are difficult to address experimentally and were therefore studied theoretically (chapter 6). Different types of pollinators were represented in the model by different 'decision rules', determining how the next plant to be visited is chosen. The choice is based on plant attractivity, which depends on the resource allocation of the plant with respect to survival, attractivity to pollinators and investment in seeds. The plant allocation pattern is genetically determined and evolves in response to the selection pressures excerted by the pollinators. Thus, the pollinator decision rule determined both the pattern of gene flow in the plant population and the optimal resource allocation of the plants with respect to survival, attractivity to pollinators and investment in seeds. The total amount of available resources of a plant is also genetically determined, by a large number of loci with deleterious recessive mutations. Due to the selection by the pollinators, a plant population becomes adapted to its principal pollinator, which may largely influence the (genetic) consequences of habitat fragmentation. Therefore, the plant-

pollinator system was allowed to adapt to equilibrium before fragmentation was applied. Starting with a large plant population, having the optimal allocation of resources for its principal pollinator type, habitat fragmentation was simulated as a reduction in population size, an increase in isolation or a shift in pollinator type. Initially, in the small remnant populations, recessive deleterious alleles became exposed due to genetic drift and inbreeding, resulting in a reduction in the amount of resources available. The allocation pattern was fixed on the short time scale of fragmentation and consequently investment in attractivity to pollinators was reduced in plants carrying many deleterious alleles. Subsequent purging of the mutation load was more efficient if the pollinators used a decision rule with high selectivity, thus reducing the impact of genetic erosion. However, highly selective pollinators induce plants to invest mainly in attractivity, resulting in very low investment in survival and seed production. This low investment in survival and seed production makes a plant population more vulnerable to demographic and environmental stochasticity, thus selective pollinators induced a high ecological vulnerability. The combination of evolutionary arguments with an analysis of the short-term genetic consequences of fragmentation showed that a high ecological vulnerability and a low genetic vulnerability are simultaneously induced by the same highly selective pollinator type.

Concluding remarks

The experiments were performed in The Netherlands, where syrphids were the main pollinators of *S. columbaria*. Syrphids transported most pollen over intermediate distances, while butterflies dispersed pollen over much longer distances. Infrequent years with large numbers of butterflies may result in an increase in long distance pollen flow, with profound effects on genetic differentiation. In France, the presence of *D. argentata* will decrease the genetic differentiation between populations of *S. columbaria*, because this specialist bee species carried pollen with a more diverse paternity and over larger distances than did Dutch syrphid flies.

A large variation was observed both in time and space. Insect abundances and estimates of pollen dispersal varied between days, years, fields and populations, which complicated the detection of general effects of fragmentation. However, the presence of variation is an inherent feature of ecological systems. Experiments without replication will easily arrive at conclusions, which are not representative for the natural situation. Therefore, experiments should not be designed to exclude all variation and arrive at a single value, but should be repeated several times to estimate the mean and variation in parameter values. Only by careful validation and the use of multiple replicates, reliable estimates can be obtained for many ecological parameters.

The net effect of a corridor depends on the balance between opposing forces. Pollen dispersal to the other patch may increase due to guidance of insects. On the other hand, pollen dispersal may decrease if insects are deterred by the corridor, if the insects remain in the corridor or if too many pollen grains get lost within the corridor. Additionally, seed set may be reduced if the presence of the corridor results in heterospecific pollen deposition. In order to separate these different aspects, three types of corridors were used: *S. columbaria* female heads (guidance and pollen loss), *S. columbaria* male heads (guidance) and *Aster*/*Origanum* (heterospecific pollen deposition, pollen loss, and guidance?). A corridor with *S. columbaria* flowers tended to increase pollen flow, while a heterospecific corridor tended to reduce pollen dispersal. For natural habitats, much longer and wider corridors are needed to connect populations and these corridors will consist of many different plant species. It may be expected that insects will frequently remain within such corridors, without ever reaching the other population and that pollen losses in the corridor will increase. Positive guidance effects of corridors on gene flow by pollen will regularly be negated by the increase in heterospecific pollen deposition and the increased residence time of insects within the corridor, resulting in increased pollen losses. Corridors between natural plant populations are presumably more effective by increasing habitat size than by increasing pollen-mediated gene flow.

The scale of the experiments was necessarily small, with much smaller distances between experimental patches than between natural populations. Nevertheless, even a distance of 25m already severely reduced pollen flow between patches, deposition in the emasculated receptor patch was only 25% of that in the source patch. As *S. columbaria* is self-compatible, such reduction in pollen deposition will not occur in (non-emasculated) natural populations and in those populations seed production will rarely be limited by pollen availability. However, an earlier study showed that a high frequency of intra-patch pollination might reduce seed quality due to inbreeding. A reduction in pollen flow may therefore result in lower population viability. Although bumblebees and syrphid flies, the main pollinators in The Netherlands, can fly long distances, transport of pollen over distances larger than 100m was rarely observed. Insect behaviour results in a leptokurtic distribution of pollen dispersal distances, implying that populations separated by larger distances are effectively isolated from each other. Consequently, the present distances between Dutch populations of *S. columbaria* (> 850m) are too large to allow gene flow by pollen and the populations can not easily be connected by the creation of corridors, making them potentially vulnerable to genetic erosion.

Samenvatting

Habitatfragmentatie, toevalsprocessen en genetische uitwisseling

In de afgelopen decennia hebben menselijke activiteiten geleid tot het verdwijnen van veel natuurlijke habitats (leefgebieden) en het versnipperen van de overgebleven gebieden. In die resterende gebieden komen vooral kleine populaties voor, die kwetsbaarder zijn voor allerlei toevalsprocessen dan grote populaties (hoofdstuk 1). Een toevallige periode met slecht weer bijvoorbeeld kan ineens het einde van een kleine populatie betekenen, terwijl van een grote groep meestal wel een paar individuen overleven. Bovendien kan in een kleine groep puur door toeval het aantal nakomelingen in een bepaald jaar gering zijn, waardoor de groep nog kleiner wordt. Dergelijke omgevings- en demografische variatie is een bekend fenomeen in kleine populaties.

Daarnaast hebben genetische toevalsprocessen zoals genetische drift een veel grotere invloed in kleine populaties dan in grote. Doordat per generatie maar een beperkt deel van de aanwezige varianten van een erfelijke eigenschap wordt doorgegeven, kunnen door toeval sommige varianten niet doorgegeven worden en verloren gaan. Als zo'n variant gunstig was, heeft dat nadelige gevolgen voor de overblijvende populatie. Was de eigenschap ongunstig, dan is het verdwijnen in eerste instantie positief. Maar ook eigenschappen die nu nadelig zijn, kunnen in de toekomst waardevol blijken als de omstandigheden zich wijzigen. Een ander effect van kleine populatiegrootte is dat langzamerhand alle individuen verwant aan elkaar worden, wat kan leiden tot inteelt. Het gezamenlijke nadelige effect van genetische drift en een verhoogde inteelt wordt genetische erosie genoemd.

Voor de bepaling van de invloed van toevalsprocessen is het erg belangrijk om te weten hoe klein een populatie precies is (hoofdstuk 1). De ernst van de genetische gevolgen van habitatfragmentatie hangt sterk af van de grootte van de overblijvende populatie. Vele factoren kunnen echter de effectieve populatiegrootte beïnvloeden, waardoor de invloed van toevalsprocessen anders is dan verwacht mocht worden op basis van het aantal aanwezige individuen in de populatie. Als bijvoorbeeld verschillende kleine populaties erfelijk materiaal met elkaar uitwisselen, dan is de effectieve grootte van die populaties groter dan het aantal individuen in de afzonderlijke populaties suggereert en zullen toevalsprocessen minder invloed hebben. Dit proefschrift gaat over de gevolgen van habitatfragmentatie voor de genetische uitwisseling tussen kleine populaties.

Genetische uitwisseling via stuifmeel in door insecten bestoven planten

Plantenpopulaties kunnen genen uitwisselen via de verspreiding van zaden en stuifmeel. Omdat zaden meestal maar kleine afstanden kunnen afleggen, kan de bijdrage van stuifmeel aan de genetische uitwisseling relatief groot zijn. Toch komen ook de meeste stuifmeelkorrels niet ver: in het algemeen legt de meerderheid van de stuifmeelkorrels maar een heel kleine afstand af en bereikt slechts een enkele stuifmeelkorrel een ver weg staande plant. Zo'n steil dalende verdeling wordt een leptokurtische verdeling genoemd (hoofdstuk 1). Hoe ver het stuifmeel precies verspreid wordt, hangt af van de manier waarop dat gebeurt. Stuifmeel kan op veel manieren verspreid worden, onder andere door wind, water of dieren. Bij veel planten wordt het stuifmeel meegenomen door bloembezoekende insecten om vervolgens weer achtergelaten te worden op andere bloemen. Het gedrag van insecten heeft een grote invloed op het patroon van stuifmeelverspreiding en de afstanden waarover stuifmeel getransporteerd wordt. Het insectengedrag hangt op zijn beurt weer af van het soort insect, de soort plant en de ruimtelijke structuur van de plantenpopulatie.

Bij versnippering van natuurlijke habitats verandert over het algemeen niet alleen de grootte van de plantenpopulatie, maar ook de dichtheid, de ruimtelijke verdeling van de planten en de afstand tot andere populaties van dezelfde soort. De effecten van dergelijke veranderingen in populatiestructuur op het gedrag van bestuivers en de gevolgen van die veranderingen voor de uitwisseling van stuifmeel vormen het onderwerp van het hier beschreven onderzoek, waarbij de plantensoort Duifkruid (*Scabiosa columbaria*) als onderzoeksobject is gekozen.

Van bezoeker naar bestuiver van Duifkruid

Omdat de insectensoort van groot belang is voor de kwaliteit van de bestuiving, en voor mogelijke veranderingen in bestuiving na fragmentatie van de populaties, werd eerst geïnventariseerd welke soorten insecten Duifkruid bezoeken en hoe effectief ze zijn voor de bestuiving. In Nederland werd Duifkruid veel bezocht door zweefvliegen en hommels, en als ze in een gebied aanwezig waren ook regelmatig door vlinders. Honingbijen en de specialistische Pluimvoetbij (*Dasypoda argentata*) werden in Nederland niet, maar in Frankrijk heel veel, op Duifkruid waargenomen. Voor de belangrijke groepen bezoekers werd de rangorde bepaald met betrekking tot een aantal aspecten van de bestuiving van Duifkruid (hoofdstuk 2).

De vliegafstand tussen opeenvolgende bloembezoeken was alleen voor vlinders regelmatig groter dan 10m, alle andere insecten vlogen vooral tussen naburige bloemhoofdjes. Voor stuifmeeluitwisseling over langere afstanden waren vlinders daarom belangrijke bezoekers. Vlinders hadden echter maar heel weinig stuifmeelkorrels bij zich en deponeerden daarvan ook maar een klein deel op vrouwelijke bloemhoofdjes. Zweefvliegen en hommels ontliepen elkaar niet veel wat betreft hun bijdrage aan de bestuiving van Duifkruid. Beide groepen verspreidden redelijk veel stuifmeel over intermediaire afstanden. Omdat ze in grote aantallen voorkwamen en met een behoorlijke snelheid bloemen bezochten, waren deze twee groepen heel belangrijk voor de bestuiving van Nederlandse Duifkruidpopulaties. Vlinders, zweefvliegen en hommels foerageerden op veel verschillende bloemsoorten en hadden slechts een laag percentage Duifkruid-stuifmeel bij zich. De bestuivingswaarde van honingbijen was vergelijkbaar met die van hommels en zweefvliegen, maar hun lading bestond vrijwel uitsluitend uit Duifkruid-stuifmeel. Pluimvoetbijen bleken met afstand de beste bestuivers te zijn, gezien de zuiverheid van hun lading en de hoeveelheid stuifmeel die ze per tijdseenheid afzetten op vrouwelijke bloemhoofdjes. Per bezoek bestoven Pluimvoetbijen slechts een fractie van de stempels in een bloemhoofdje, maar ze bezochten erg veel hoofdjes per minuut. Door die hoge bezoeksnelheid

werden de bloemhoofdjes zo vaak bezocht dat in korte tijd toch alle stempels bestoven werden, met bovendien een zeer gevarieerd vaderschap van de zaden. Pluimvoetbijen vlogen ook nog eens redelijke afstanden, zodat de genetische uitwisseling in de (Franse) Duifkruidpopulaties frequent was. In Nederland komt de Pluimvoetbij niet voor. Nederlandse Duifkruidpopulaties waren daarom voor de kwantiteit en kwaliteit van hun bestuiving afhankelijk van meerdere soorten bestuivers, die elk minder efficiënt waren dan Pluimvoetbijen.

Onderzoek naar stuifmeelverspreiding

In een serie experimenten werden de gevolgen van habitatfragmentatie voor de verspreiding van stuifmeel onderzocht. Om het relatieve belang van verschillende aspecten van habitatfragmentatie te kunnen vergelijken werd in alle proeven gebruik gemaakt van dezelfde standaard proefopstelling. Deze standaardopstelling bestond uit een lijnvormige Duifkruid-populatie in een weiland in Assen. De populatie werd gevormd door drie kleine, maar evengrote groepjes planten (‘patches’), die 25m uit elkaar lagen. De bestuiving geschiedde door de van nature voorkomende insecten.

In veel proeven (behalve die met genetische merkers, zie verderop) functioneerde de middelste patch als donor van stuifmeel. De twee buitenste patches werden geëmasculeerd en functioneerden als receptor van stuifmeel. Emasculatie wil zeggen dat de meeldraden en helmhokken, met daarin het stuifmeel, van alle bloemen verwijderd werden. Omdat er bij Duifkruid voortdurend nieuwe helmhokken opengaan, werd het verwijderen elk half uur herhaald. Een geëmasculeerde receptor patch kon dus zelf geen stuifmeel meer verspreiden, zodat al het stuifmeel dat daar werd gevonden uit de donor patch (de middelste patch) kwam.

Variatie tussen proefvelden, dagen en jaren is een inherente eigenschap van experimenten onder (semi-)natuurlijke omstandigheden. Niet alleen kan het aantal insecten en hun soortensamenstelling sterk variëren (hoofdstukken 3 en 5), extra variatie kan ook een gevolg zijn van verschillen in de aanwezigheid van andere bloeiende planten in de omgeving, van de emasculatie-behandeling en van individuele variatie tussen bestuivers in stuifmeellading en foerageergeschiedenis. Daarom werd ook variatie in stuifmeelverspreiding verwacht (zie ook de discussie bij hoofdstukken 3 en 4). Om ondanks de grote variatie toch conclusies te kunnen trekken over de gevolgen van fragmentatie voor de bestuiving werden alle experimenten een aantal keren herhaald van augustus tot begin oktober, en werden de resultaten gecombineerd. Hoewel de variatie groot was en het aantal herhalingen beperkt, konden toch een aantal duidelijke conclusies worden gevonden (zie onder de betreffende experimenten).

Het meten van genetische uitwisseling via stuifmeel

Er bestaan verschillende methoden om de hoeveelheid stuifmeeluitwisseling tussen populaties te schatten. Ze variëren van het volgen van bloembezoekende insecten, via de verspreiding van fluorescerend poeder (een veelgebruikt analoog van stuifmeel) tot het bepalen van de verspreiding van zeldzame genetische kenmerken. Omdat elke methode voor- en nadelen heeft, werd eerst in de standaard proefopstelling met Duifkruid onderzocht in hoeverre de resultaten van een aantal van deze technieken overeenkwamen.

Op vijf dagen, verspreid over augustus en september, werd tegelijkertijd de verspreiding van stuifmeelkorrels, fluorescerend poeder en genetische merkers gevolgd (hoofdstuk 3). Het volgen van insecten was in deze proef niet goed mogelijk, omdat het terugzien van gemerkte zweefvliegen, die heel belangrijk waren in ons systeem, vrijwel geen succes had. Voor het volgen van de verspreiding van genetische merkers werd eerst van alle planten onderzocht welke

variant ze bezaten van een bepaald enzym (een eiwit dat een specifieke chemische reactie bevordert in de cel). In de proef bestond elke patch uit planten met een andere variant. Vervolgens werd in de zaden onderzocht welke varianten van het enzym aanwezig waren. Uit de in het zaad aanwezige genetische varianten werd afgeleid in welke patch de vaderplant had gestaan en dus hoe ver het stuifmeel verspreid was. Tegelijkertijd kreeg elke patch een andere kleur fluorescerend poeder, aangebracht op de helmhokken. Na de proef werd onder een fluorescentie-microscoop gekeken welke kleuren poeder in welke hoeveelheden op de stempels gedeponeerd waren, waaruit het patroon van poederverspreiding bepaald werd.

Er was een grote variatie in transport van genetische merkers, poeder en stuifmeelkorrels tussen proefvelden en tussen dagen, maar als de resultaten van alle dagen gecombineerd werden bleken de verspreidingspatronen van stuifmeelkorrels, fluorescerend poeder en genetische merkers vrijwel identiek. Het transport van stuifmeelkorrels werd echter bepaald in geëmasculeerde patches, waarin binnenkomend stuifmeel geen concurrentie van stuifmeel uit de eigen patch ondervond. Hierdoor lagen schattingen van de genetische uitwisseling over een bepaalde afstand wat hoger indien ze gebaseerd waren op stuifmeeltellingen ten opzichte van schattingen die gebaseerd waren op fluorescerend poeder of genetische merkers (hoofdstukken 3 en 4). Voor onze experimenten waren dergelijke kwantitatieve verschillen niet belangrijk, omdat steeds het stuifmeeltransport naar de receptor patch op 25m (controle patch) werd vergeleken met dat naar de andere receptor patch, die een behandeling kreeg. Hiervoor was de relatieve hoeveelheid stuifmeelverspreiding belangrijk en zo'n kwalitatieve meting kon met alle drie methoden gedaan worden, omdat de vorm van de verspreidingscurve hetzelfde was. Aangezien het bepalen van de verspreiding van fluorescerend poeder en genetische merkers relatief veel tijd (en voor de genetische merkers geld) kostte, werd er voor de meeste proeven gekozen voor het tellen van stuifmeelkorrels in receptor patches met geëmasculeerde planten. Bij Duifkruid kan dat relatief gemakkelijk gedaan worden, want de stuifmeelkorrels zijn zo groot (50-70 µm) dat ze met een loep (20x) goed te zien waren. Een extra voordeel van stuifmeel tellingen is dat het verloop van de proef gedurende de dag gevolgd kon worden en de resultaten direct aan het eind van de dag bekend waren, zodat eventueel volgende proeven nog bijgesteld konden worden.

Bestuiving in gefragmenteerde habitats

Bij fragmentatie nemen de afstanden tussen populaties toe en regelmatig zullen er allerlei hindernissen voorkomen tussen de overblijvende populaties. Beide kunnen een belemmering vormen voor bloembezoekende insecten en de uitwisseling van stuifmeel tussen populaties beperken. Deze aspecten van fragmentatie werden in aparte experimenten onderzocht. Hiervoor werd de afstand van de receptor patch tot de donor patch vergroot tot maximaal 200m of werd er een camouflagenet als hindernis tussen de donor- en receptor patch geplaatst (hoofdstuk 4). De laatste jaren wordt veel gesproken over het aanleggen van verbindingszones, zogenaamde corridors, tussen fragmenten natuurgebied. Om te onderzoeken of corridors daadwerkelijk effectief kunnen zijn voor het bevorderen van stuifmeeluitwisseling werd tussen de donor patch en de receptor patch een lijn van bloeiende bloemen geplaatst (hoofdstuk 5). Tot slot werd in een theoretische studie geprobeerd om inzicht te krijgen in mogelijke (genetische) gevolgen van een verschuiving in de insectensamenstelling als gevolg van habitatfragmentatie.

Isolatie door afstand

Isolatie door afstand leidde tot een sterke verlaging van het stuifmeeltransport. Al op 25m afstand werd in een patch met geëmasculeerde planten nog maar een kwart van de hoeveelheid stuifmeel gevonden ten opzichte van de donor patch. Op 200m afstand werd nauwelijks stuifmeel aangetroffen (hoofdstuk 4). Transport van fluorescerend poeder en genetische merkers daalde zelfs sterker met afstand dan de verspreiding van stuifmeelkorrels, daarvan werd nog maar 10% gedeponeerd in een patch op 25m van de donor patch (hoofdstukken 3 en 4). Schattingen van de hoeveelheid genetische uitwisseling op basis van stuifmeeltellingen leidden dus tot een overschatting van de werkelijke uitwisseling van genetische varianten. De zaadproduktie liet ook een sterke daling zien met toenemende afstand tot de donor patch, hoewel minder scherp dan de daling van het aantal stuifmeelkorrels. Dat werd mogelijk veroorzaakt door de hoge stuifmeeldepositie per stempel in de proeven, terwijl vier stuifmeelkorrels per stempel al voldoende zijn voor maximale zaadproduktie en meer stuifmeel geen extra zaden opleverde (hoofdstuk 3).

Barrières en corridors

Een camouflagenet is gebruikt om de werking van barrières na te bootsen. Door het net was de achterliggende patch niet meer zichtbaar. Ondanks meerdere herhalingen was de variatie in stuifmeeluitwisseling tussen proefvelden, dagen en jaren te groot om een eenduidige conclusie te kunnen trekken (hoofdstuk 4). Hoewel de resultaten soms zelfs tegenstrijdig waren, was het camouflagenet echter duidelijk geen hindernis voor het transport van stuifmeel. Dit laat zien dat objecten die door mensen als hindernis ervaren worden, dat niet hoeven te zijn voor bestuivers en dat men dus erg voorzichtig moet zijn met uitspraken over potentiële barrières.

Er wordt veel gesproken over de aanleg van verbindingszones om de uitwisseling te bevorderen tussen kleine natuurgebieden. Voor een aantal dieren is bekend dat corridors migratie mogelijk maken, maar over de effectiviteit met betrekking tot planten is weinig bekend. Als de verspreiding van stuifmeel afhangt van bestuivers zal de soortensamenstelling van de bloemen in de corridor erg belangrijk zijn, net als mogelijkerwijs hun ruimtelijke verdeling in de corridor. In een serie experimenten werden de ruimtelijke verdeling en de soorten bloemen in de corridor gevarieerd en hun effecten op de stuifmeeluitwisseling bepaald (hoofdstuk 5).

De soort bloemen in de corridor had effect op zowel het gedrag van de insecten als op de verspreiding van stuifmeel. Een corridor met vrouwelijke Duifkruid-bloemhoofdjes leidde tot een groot verlies van stuifmeel op de bloemen in de corridor. Desondanks was er een tendens tot grotere stuifmeeldepositie in de patch, die door de corridor met de donor patch verbonden was, ten opzicht van de controle patch. Geleiding van insecten langs de corridor naar de andere patch was blijkbaar belangrijker dan het verlies aan stuifmeel in de corridor.

In een corridor met Duifkruid vlogen insecten kleinere afstanden tussen opeenvolgende bloembezoeken dan in een corridor met aster. Met grotere vliegafstanden in een aster-corridor werden er minder bloemen bezocht voordat de andere patch bereikt is en zou het verlies aan stuifmeel in de corridor lager moeten zijn, waardoor er meer stuifmeel overblijft om te deponeren in de receptor patch. Toch was er een trend naar verlaagde stuifmeeldepositie in de patch die door een aster-corridor verbonden is met de donor patch. Mogelijk werden veel insecten afgeschrikt door de andere bloemsoort in de corridor en waren er daarom minder insecten die de overtocht volbrengen. Een niet onbelangrijk neveneffect van een aster-corridor was de grote hoeveelheid aster-stuifmeel die in de Duifkruid-patch aankwam. Dit soortvreemde stuifmeel uit de corridor kan de zaadproduktie van Duifkruid negatief beïnvloeden. De verspreiding van stuifmeel hing in deze experimenten, met een gering aantal herhalingen per variant, niet af van de ruimtelijke verdeling.

Invloed van bestuiver-plant interacties

Verschillende soorten bestuivers reageren verschillend op fragmentatie van de plantenpopulatie. Drie zweefvliegsoorten bijvoorbeeld, hadden verschillende vliegafstanden in dezelfde corridor. Hierdoor zal ook de hoeveelheid Duifkruidstuifmeel en soortvreemd stuifmeel, die aankomt in de patch aan de andere kant van een corridor, veranderen (hoofdstuk 5). Naast zulke variatie in reactie, zullen ook regelmatig insectensoorten verdwijnen, waardoor de soortensamenstelling veranderd als een plantenpopulatie gefragmenteerd raakt. Dergelijke veranderingen zijn echter moeilijk experimenteel te onderzoeken en werden daarom in een computersimulatie bestudeerd (hoofdstuk 6).

In de simulatie had een plant erfelijke eigenschappen voor de investering van energie in ‘attractiviteit’ voor bestuivers, investering in overleving en investering in zaadproduktie. Bovendien had een plant een hele groep genen die gezamenlijk het totaal aan beschikbare energie bepaalden. Van elk gen waren 2 varianten aanwezig, een normale en een variant voor lagere energie. Deze laatste, nadelige, variant was recessief, dat wil zeggen dat zo’n variant pas tot een lagere energie-beschikbaarheid leidt als hij met eenzelfde type als hijzelf gecombineerd wordt en niet in combinatie met een normale variant. Genetische erosie van deze groep genen leidde tot een toename van het aantal ‘dubbel’ ongunstige varianten en dus een lagere beschikbaarheid van energie. De bestuivers werden abstract nagebootst door een ‘beslissingsregel’ op grond waarvan ze kozen welke plant als volgende bezocht zou worden. Als basis voor de keuze functioneerde de attractiviteit van de plant; bestuivers konden meer of minder onderscheid maken tussen planten met verschillende attractiviteiten. Daarmee bepaalde de beslissingsregel het patroon van stuifmeelverspreiding en ook de optimale verdeling van energie over attractiviteit, overleving en zaadproduktie. Op evolutionaire tijdschaal zal een plantenpopulatie aangepast raken aan de belangrijkste bestuivers en dat zal invloed hebben op de te verwachten gevolgen van habitatfragmentatie. Daarom kreeg elke combinatie van plant en bestuiver eerst de gelegenheid om aan elkaar aangepast te raken, voordat fragmentatie werd toegepast.

Voor deze adaptatie werd begonnen met een grote populatie, waarin de optimale verdeling van energie over attractiviteit, zaadproduktie en overleving werd bepaald, afhankelijk van de selectiviteit van de aanwezige bestuivers. Eenmaal aangepast werd de plantenpopulatie onderworpen aan habitatfragmentatie, die gemodelleerd werd als een verkleining van de populatiegrootte, een toename van de isolatie of een verschuiving naar een ander bestuiverstype. Kort na fragmentatie leidden genetische drift en inteelt tot vele individuen met een groot aantal dubbele nadelige varianten, die minder energie beschikbaar hebben. Omdat op de korte tijdschaal waarop fragmentatie belangrijk is, geen adaptatie mogelijk is van de energie-verdeling, hadden deze planten ook een lage attractiviteit voor bestuivers. Al snel steeg de beschikbaarheid van energie weer, als gevolg van het verdwijnen van de individuen met veel nadelige varianten door selectie (‘purging’). Dit uitselecteren van nadelige varianten ging het snelst als de bestuivers sterk selecteerden op de attractiviteit van de planten. Naast dit positieve effect met betrekking tot genetische erosie, zorgden deze selectieve bestuivers voor een erg scheve optimale verdeling van energie. Als de bestuivers sterk selecteerden, gingen de planten vrijwel alle energie investeren in attractiviteit en bleef er slechts weinig energie over voor overleving en zaadproduktie. Daardoor werden de planten kwetsbaar voor toevalsvariatie in voortplantingssucces en voor omgevings- en weersinvloeden (‘ecologische’ kwetsbaarheid). De combinatie van evolutionaire argumenten met een analyse van de genetische effecten van fragmentatie op de korte termijn liet zien dat een hoge ecologische kwetsbaarheid en een lage genetische kwetsbaarheid beide tegelijk konden ontstaan als gevolg van hetzelfde (selectieve) type bestuiver.

Ter afsluiting

De experimenten zijn gedaan in Nederland, waar zweefvliegen de belangrijkste bestuivers van Duifkruid zijn. Zweefvliegen transporteren stuifmeel over intermediaire afstanden, terwijl vlinders veel grotere afstanden overbruggen. Als er af en toe een jaar is waarin er veel vlinders zijn, wordt er veel meer stuifmeel over grote afstanden uitgewisseld dan in de experimenten gemeten is. Zo’n relatief bijzonder jaar kan grote gevolgen hebben voor de genetische differentiatie tussen populaties. In Frankrijk zal de genetische differentiatie tussen Duifkruidpopulaties lager zijn dan in Nederland, omdat daar de Pluimvoetbij voorkomt. Deze gespecialiseerde bijensoort transporteert stuifmeel van veel meer verschillende vaderplanten en over grotere afstanden dan de Nederlandse zweefvliegen.

Er treedt vaak veel variatie in ruimte en tijd op in dit type experimenten, wat het soms moeilijk maakte om algemene conclusies te trekken uit de resultaten. Aantallen en soorten insecten en de uitwisseling van stuifmeel vertoonden allemaal veel variatie tussen dagen en jaren en ook tussen proefvelden en natuurlijke populaties. Variatie is echter een onvermijdelijke eigenschap van ecologische systemen. Experimenten zonder herhalingen lopen een grote kans om een niet-representatief resultaat te vinden. De opzet van experimenten moet er daarom ook niet op gericht zijn om variatie uit te bannen en een enkel getal als antwoord te geven, maar juist om de variatie in kaart te brengen. Alleen met een goede ijking van methoden en het gebruik van voldoende herhalingen ontstaat inzicht in de variatie en kan er een betrouwbaar beeld gekregen worden van veel ecologische parameters.

Het netto effect van een corridor hangt af van de balans tussen een aantal tegenstrijdige effecten. Enerzijds kan de stuifmeeluitwisseling tussen patches toenemen doordat insecten langs de corridor naar de andere patch geleid worden. Anderzijds kunnen insecten ook juist afgeschrikt worden door een corridor, of juist blijven foerageren in de corridor in plaats van door te vliegen naar de andere patch. Ook kan er in een corridor veel Duifkruidstuifmeel achterblijven en er kan soortvreemd stuifmeel worden meegenomen wat vervolgens afgezet wordt in de volgende patch. In de experimenten is gekozen voor een proefopzet waarin een aantal van deze effecten konden worden onderscheiden. Een Duifkruid-corridor resulteerde in een licht verhoogde stuifmeel-uitwisseling, terwijl een aster-corridor leidde tot een lager stuifmeeltransport. Om verbindingen tussen natuurlijke populaties te kunnen maken zijn echter veel langere en bredere corridors nodig, met meer planten(soorten), dan hier gebruikt. Het mag verwacht worden dat insecten relatief vaker zullen blijven foerageren in dergelijke grote en gevarieerde corridors in plaats van door te vliegen en dat minder stuifmeel de andere populatie zal bereiken. Positieve geleidings-effecten op de stuifmeeluitwisseling zullen regelmatig teniet gedaan worden door de toename van negatieve effecten zoals stuifmeelverlies in de corridor en de depositie van soortvreemd stuifmeel. Positieve effecten van corridors tussen natuurlijke plantenpopulaties zijn waarschijnlijk eerder te verwachten in de vorm van vergroting van het leefgebied dan in de vorm van verhoogde (genetische) uitwisseling.

De experimenten vonden plaats op een veel kleinere schaal dan fragmentatie van natuurlijke habitats. Toch bleek dat ook een geringe afstand van 25m al resulteerde in een sterk verminderde stuifmeeluitwisseling tussen patches. In de proeven met geëmasculeerde patches leidde dat tot een afname van de zaadproduktie. Omdat Duifkruid zelfbestuivend is, zal de zaadproduktie in natuurlijke populaties niet gauw verminderen door een tekort aan stuifmeel, want daar is veel lokaal stuifmeel uit de eigen populatie beschikbaar. Echter, een vermindering van de stuifmeeluitwisseling kan wel de zaadkwaliteit beïnvloeden en daarmee de levensvatbaarheid van de populatie op de lange termijn. Uit eerder onderzoek is namelijk gebleken dat de zaden van tussen-populatie-kruisingen een hogere overleving en voortplanting hebben dan zaden van binnen-populatie-kruisingen of zaden van zelfbestuiving. Uit de gevonden sterke afname van de stuifmeeluitwisseling met toename van de afstand, blijkt dat hommels en zweefvliegen, de belangrijkste bestuivers van Duifkruid in Nederland, grotere afstanden niet vaak overbruggen, hoewel ze het wel kunnen. Het meeste stuifmeel verspreidden ze binnen 100m van de bron. De leptokurtische verdeling van verspreidings-afstanden impliceert dat er geen stuifmeel uitgewisseld wordt tussen populaties die door grotere afstanden van elkaar gescheiden zijn. Ook met corridors zullen grotere afstanden waarschijnlijk niet effectief overbrugd worden door stuifmeel. Dat wil zeggen dat de Nederlandse Duifkruidpopulaties, die nu meer dan 850m van elkaar verwijderd zijn, te ver uit elkaar liggen om op een natuurlijke manier genetische informatie uit te kunnen wisselen.