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 argentina 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 25 m, 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 emascula

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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 attractiveness, which depends on the resource allocation of the plant with respect to survival, attractiveness to pollinators and investment in seeds. The plant allocation pattern is genetically determined and evolves in response to the selection pressures exerted 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, attractiveness 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.