University of Groningen

Biodiversity and pollination

Hoffmann, Frank

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2005

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Hoffmann, F. (2005). *Biodiversity and pollination: Flowering plants and flower-visiting insects in agricultural and semi-natural landscapes*. s.n.

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 03-06-2022

Biodiversity and pollination

Flowering plants and flower-visiting insects in agricultural and semi-natural landscapes

This research was financially supported by a grant from the Netherlands Organisation for Scientific Research - Earth and Life Sciences (NWO-ALW 014-22-043).
The research was carried out at the Community and Conservation Ecolgy Group of the University of Groningen, PO Box 14, 9750 AA Haren, the Netherlands.

Layout and graphics:	Dick Visser
Photos:	by author, Manja Kwak (front cover (partially) p.20, p.134, p.218) and R. Frankot (p.222).
Printed by:	Febodruk B.V. Enschede

ISBN electronic version: 90-367-2444-9

ISBN: 90-367-2443-0

RIJKSUNIVERSITEIT GRONINGEN

Biodiversity and pollination

Flowering plants and flower-visiting insects in agricultural and semi-natural landscapes

PROEFSCHRIFT

ter verkrijging van het doctoraat in de
Wiskunde en Natuurwetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus, dr. F. Zwarts,
in het openbaar te verdedigen op
vrijdag 2 december 2005
om 14:45 uur

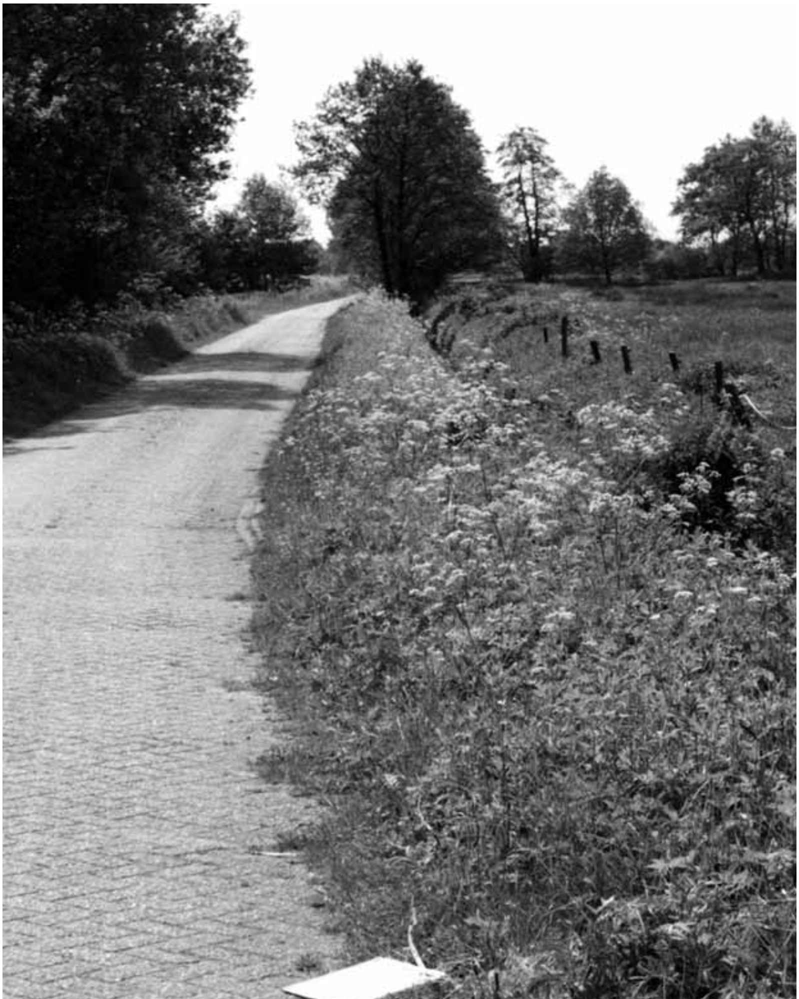
door

Frank Hoffmann

geboren op 4 februari 1974
te Zeven

Promotor:	Prof. dr. J. van Andel
Copromotor:	Dr. M. M. Kwak
Beoordelingscommissie:	Prof. dr. N.M. Waser Prof. dr. W.H.O. Ernst Prof. dr. E. van der Meijden

Contents



1

Introduction

Biodiversity and ecosystem functioning

The Millennium Ecosystem Assessment (MA) Synthesis Report (UNEP 2005) unambiguously showed to the world that during the past 50 years humans have changed ecosystems in a way unprecedented in any other period of human history. The planet's biodiversity has declined, and population sizes and ranges of the majority of species across many taxonomic groups are currently declining. It was calculated that humans may have increased the extinction rate to as much as 1,000 times over the background extinction rates. The degradation of ecosystems could become significantly worse if policies and practices are not changed, with negative effects on the gains from ecosystem services (e.g. fresh water, food, air, regulation of climate and pests, and esthetical and spiritual services) for future generations. There is established evidence that changes in ecosystems are increasing the likelihood of non-linear (abrupt) and cascading further changes in ecosystems. However, the evidence is incomplete according to the MA. Connected to this, the secretary general of the United Nations, Kofi Annan, said some important words when the MA was launched on March 30th, 2005: "Only by understanding the environment and how it works can we make the necessary decisions to protect it." In other words, scientific knowledge about ecosystem functioning is a prerequisite for developing new policies.

The MA mentioned biodiversity loss as one cause in a complex of causes for changes of ecosystem functioning and declining ecosystem services (UNEP 2005). Biodiversity in the sense of species richness may play a crucial role for maintaining ecosystem processes and therefore also for maintaining biodiversity itself, but evidence for this is poor (Loreau *et al*. 2002). Decreased levels of biodiversity in an ecosystem may have a negative effect on the stability of that ecosystem (De Leo & Levin 1997; Lehman & Tilman 2000; McCann 2000; Loreau *et al*. 2002), may decrease the productivity of vegetation (Lehman & Tilman 2000; Tilman *et al*. 2001), affect food web structure (McCann 2000; Dunne *et al*. 2002), and lower the resistance to (harmful) species invasions (Kennedy *et al*. 2002). One problem concerning research about the importance of biodiversity is the lack of consensus about the meaning of the terms ecosystem stability, or even functioning (De Leo & Levin 1997; McCann 2000). Furthermore, most studies describing effects of biodiversity are theoretical, or they are field studies that were originally meant for other research questions (Loreau *et al*. 2002).

There are three classes of hypotheses addressing the role of biodiversity for ecosystem functioning (Loreau *et al*. 2002): firstly, species are primarily redundant or at least partially substitutible ("redundancy hypothesis"), i.e. all species have approximately equal importance for the ecosystem, and the removal of a species is compensated for by other species. Secondly, species are primarily singular or make unique contributions to ecosystem functioning ("keystone-species hypothesis"), i.e. ecosystem functioning is regulated by few dominant or key-stone species. Thirdly, species effects are context-dependent ("context-dependence hypothesis"), i.e. the effects of losses or additions of species depend on specific conditions, such as community composition and site fertility.

In an ecosystem many processes take place simultaneously; therefore studying the effect of biodiversity on one particular process with a known function may provide better results than trying to understand effects on the whole ecosystem. One ecological process is biotic pollination, a process involving two parties or communities (plants and animals), that can mutually benefit from each other (Proctor 1978; Fægri & van der Pijl 1979). Animals actively or passively transfer pollen grains, containing the male gametes, from the stamina to the stigmas, where the pollen grains can germinate and fertilise the female gamete in the ovule. Animals find food, shelter or pheromones in the flowers, and many animal species are dependent on flowers. Great diversity exists in the way plants have adapted their flowers to insect visitation, and likewise among the animals that visit the flowers and are potential pollinators (Loew 1895; Knuth 1898; Proctor 1978; Fægri & van der Pijl 1979).

The loss of species has not spared plants and their pollinators; many plant and animal species have declined or gone extinct, and thousands are threatened to disappear (Buchmann & Nabhan 1996). Great concern exists for the negative consequences this may have for the reproduction of wild plants and crops (Buchmann & Nabhan 1996; Kearns & Inouye 1997; Fisher 1998; Kearns *et al*. 1998; Cox & Elmqvist 2000; Kremen & Ricketts 2000; Roubik 2000; Cane & Tepedino 2001; Spira 2001; Wilcock & Neiland 2002). Continued loss of pollinator species may lead to a worldwide "pollination crisis" (Buchmann & Nabhan 1996). This may also endanger the human food supply, as many crops need insect pollination for the produce to develop, or seeds for next generations of vegetative crops (Richards 2001). For example, in the EU up to 83% of the 264 species grown as crops are animal-pollinated (Williams 1996). To date, there are no studies that showed the exact role of biodiversity for pollination (Rathcke & Jules 1993; Kwak *et al*. 1998); either only data are given about species richness without effects on pollination or seed set, or studies focus on one or a few plant and animal species at a time (Kwak *et al*. 1998).

The main subject of this thesis will therefore be the importance of biodiversity for the pollination of entomophilous plants. It will include different aspects from landscape and community level to pollination and seed set at the level of individual species or patch.

The nature of biotic pollination

There is a tremendous diversity of pollination systems, and there are great differences in the degree of specialisation for pollinators among plants or for flowers among animals (Proctor 1978; Fægri & van der Pijl 1979; Waser *et al*. 1996). Flowers can be very simple and accessible to many flower visitor species, or very complicated and limit the number of possible visitor species. There are even plant species that deceive insects by mimicry, or plant species that kill their pollinators in trap-like flowers (Fægri & van der Pijl 1979). Pollinators can be as different as insects, birds, bats and other small mammals (Proctor 1978; Fægri & van der Pijl 1979), and even lizards (Traveset & Sáez 1997; NyHagen *et al*. 2001). Pollination systems can be classified into types of plant species with functionally similar flowers and similar pollinator compositions (guilds). These types are called pollination syndromes (Proctor 1978; Fægri & van der Pijl 1979; Armbuster *et al*. 2000;

Ollerton & Watts 2000). Insects are the dominant pollinators on earth (Fægri & van der Pijl 1979); at least 70 percent of all angiosperms are insect-pollinated (Kearns & Inouye 1997). Insects are the only pollinators of importance in northern Eurasia (Ellis & Ellis-Adam 1993; Memmott 1999; Elberling & Olesen 1999; Totland *et al*. 2000), including the research area of this thesis, which is situated in the Netherlands. Therefore, in this thesis only pollination by insects will be studied.

The majority of plant-pollinator interactions are of a generalised nature, and only the minority of species interactions are specialised (Jordano 1987; Ellis & Ellis-Adam 1993; Waser *et al*. 1996; Memmott 1999). One-to-one relationships between single plant and animal species are extremely rare, particularly in temperate climates (Kwak *et al*. 1998). One should be aware that the degree of specialisation can be regarded in two ways: evolutionary and ecological specialisation (Armbuster *et al*. 2000). Evolutionary specialisation is a process with a direction, i.e. from many to less pollinating taxa. The ecological specialisation of a species is a state, referring to having few pollinators relative to other plant species, or visiting few functional types of flowers compared to other animals in case of the flower visitors. Furthermore, specialisation and generalisation are not a dichotomy, but a continuum (Waser *et al*. 1996; Armbuster *et al*. 2000; Johnson & Steiner 2000).

The distribution of interactions between plants and flower visitors is highly asymmetrical (Jordano 1987; Bronstein 1995; Waser *et al*. 1996; Memmott 1999; Olesen & Jordano 2002). Many specialist plant species are visited by generalist insects, whereas many specialist insects visit plant species that are also visited by (many) generalist insects. Flowers may show specialised traits, but are paradoxically visited by a large number of species. It may be that only a small proportion of the visitors are actual pollinators, functioning as a selective force (Johnson & Steiner 2000; Ollerton & Watts 2000). Another explanation may be that flowers can also be adapted to relatively less effective pollinators when this adaptation causes little loss in the fitness contribution of a more effective pollinator (Aigner 2001). Furthermore, both levels are dominated by a small group of abundant plant and animal species (Olesen & Jordano 2002).

Biodiversity and pollination

Biotic pollination involves two parties: plants and animals (Proctor 1978; Fægri & van der Pijl 1979). Therefore the diversity of both these two levels may be important for pollination. Changes in species richness and functional diversity (the number of groups of similar species) of plants and insects, and changes in plant population size and structure can affect pollination (Rathcke & Jules 1993; Buchmann & Nabhan 1996; Kremen & Ricketts 2000). Pollination consists of quantity components, i.e. the number of visitor species per plant species and the number of visits and pollinations a plant or flower receives, and quality components, i.e. con- and heterospecific pollen deposition (Kwak *et al*. 1998). For many plant species a decline in pollination quantity and quality will result in a lower seed set (Ågren 1996; Kearns & Inouye 1997; Kwak *et al*. 1998; Steffan-Dewenter & Tscharntke 1999; Tomimatsu & Ohara 2002) and/ or inbreeding (van Treuren *et al*. 1993;

Oostermeijer *et al*. 1994; Kwak *et al*. 1998; Velterop 2000; Luijten 2001; Mustajärvi *et al*. 2001), and is thus detrimental to the survival of plant populations. In many but not all plant species the negative effects of reduced (cross) pollination can be postponed by self-pollination (autogamy), long-term seed banks or clonal propagation (Kearns & Inouye 1997; Spira 2001).

Plant diversity (species richness and composition) can have various effects on plant-pollinator interactions. Firstly, the number of plant species is positively correlated to the number of flower-visiting insect species and individuals (Corbet 1997; Bäckman & Tiainen 2002; Collinge *et al*. 2003; Potts *et al*. 2003). The presence of other plant species can also be facilitating by increasing the visitation rate, i.e. the number of visits per unit time, particularly for plant species with low densities or in small patches (Thomson 1978; Schemske 1981; Kwak 1988; Laverty 1991). Plants can also benefit from each other in time; insects often have longer phenological time spans than the flowering period of a particular plant species and therefore need several sequentially flowering plant species during their life span (Bronstein 1995). This is particularly important for social species such as bees; bumblebee colonies can grow faster when early-flowering plants are close to the colony, producing a larger number of workers that can pollinate species flowering later (Kwak *et al*. 1998).

Plants can affect each other also negatively: competition between plant species acts via the number of visitors, visitation rate, heterospecific pollen deposition and pollen loss, resulting in reduced seed set (Waser 1978a; Campbell & Motten 1985; Campbell 1985b; Jennersten & Kwak 1991; Armbuster & McGuire 1991; Kwak *et al*. 1998; Brown *et al*. 2002) or reduced pollen flow distance (Campbell 1985a). The balance between competition and facilitation depends on the plant species, community composition and flower abundance and density (Kwak *et al*. 1998). Competitive or facilitative interactions will most likely occur between plant species with a shared pollinator fauna (Waser 1978b, 1979; Rathke 1983; Feinsinger 1987).

The chance that a certain plant species is visited by its appropriate pollinator(s) may increase as insect species richness is higher (Corbet 1997). The loss of pollinator species will be detrimental for many plant species by reducing pollination and seed set (Rathcke & Jules 1993; Buchmann & Nabhan 1996; Kwak *et al*. 1996; Corbet 1997; Tepedino *et al*. 1997; Fisher 1998; Kearns *et al*. 1998; Kremen & Ricketts 2000; Spira 2001). However, as the majority of species are generalists and the nature of plant-pollinator interactions is more like that of a complex foodweb (Memmott 1999; Corbet 2000b; Olesen & Jordano 2002; Memmott *et al*. 2004), the disappearance of a single species at one of the interacting levels does not directly lead to an extinction at the other level (Kearns *et al*. 1998). To plants and insects the significance of the loss of partners depends on whether the pollination relationship is facultative or obligate (Kearns & Inouye 1997; Kearns *et al*. 1998). Generalist plant species may be resilient to pollinator species loss, because pollinator species that have disappeared may be replaced with other species (Rathcke & Jules 1993; Waser *et al*. 1996; Spira 2001). However, the pollination quality and availability of alternative visitors determines the exchangeability (Rathcke & Jules 1993; Kwak 1994b; Kwak *et al*. 1998). Plant species that depend on few or single pollinator species are said to be the most vulnerable, as the loss of pollinator species may leave few or no alternatives (Rathcke & Jules 1993; Bronstein 1995; Kearns & Inouye 1997; Kwak *et al*. 1998; Johnson & Steiner 2000; Spira 2001). Data showing whether plant species with various degrees of specialisation differ in their vulnerabilities to pollinator loss are currently lacking.

In the end, not the pollinator species, but the choices made by individual flower visitors are the ultimate determinants for the pollination of plants (Kunin 1993; Kwak *et al*. 1998). Flower constancy of individual visitors is affected by the availability of rewards (Goulson 1999; Hill *et al*. 2001) and by the composition of a flower community (Waser 1986; Chittka *et al*. 1997; Slaa & Biesmeijer 2003). A low flower constancy can cause pollen loss to other plant species and deposition of heterospecific pollen on the stigmas of the target species (Rathke 1983; Campbell 1985a). Heterospecific pollen deposition may not be caused by the diversity of a plant community per se, but by the densities and abundances of the component plant species in that community (Feinsinger *et al*. 1986). The effects of heterospecific pollen deposition on seed set are reported to be neutral (Schemske 1981; Campbell & Motten 1985; Kwak & Jennersten 1986) or negative (Waser 1978b; Randall & Hilu 1990; Kunin 1993; Kwak & Bergman 1996; Brown & Mitchell 2001).

The outcome of mutual interactions between (groups of) species such as in pollination is context-dependent, i.e. costs and benefits of the partners vary in space and time and are influenced by various ecosystem characteristics (Bronstein 1994). As most plant-pollinator interactions are of a generalised and facultative nature, plants and flower visitors are mutualists at community rather than at species level (Armbuster *et al*. 2000). Biodiversity is only one aspect that interacts with other aspects in a habitat and landscape context that affects the pollination mutualism. Biodiversity decline itself often has habitat fragmentation as one of its major causes (Jennersten 1988; Rathcke & Jules 1993; Buchmann & Nabhan 1996; Steffan-Dewenter & Tscharntke 1997; Kearns *et al*. 1998; Kwak *et al*. 1998; Kremen & Ricketts 2000; Cane 2001; Fahrig 2003; Ashworth *et al*. 2004; UNEP 2005). Population sizes and flower densities of individual plant species are, among others, related to habitat fragmentation, and also interact with species richness (Kearns *et al*. 1998). Plant species in small and fragmented populations may be particularly vulnerable to pollination changes (Oostermeijer *et al*. 1994, 2000; Washitani 1996; Kwak *et al*. 1998; Luijten *et al*. 2000).

Reviewing the literature stated above, no complete hypotheses about the effects of biodiversity on pollination can be found, only a number of ideas distributed over several texts. At first sight it may appear from authors warning for the effects of species decline, that every species extinction will be detrimental. This idea belongs to the "redundancy" type of hypotheses (see above), where all species in an ecosystem or community are equally important. However, with the asymmetric distribution of interactions in mind, it is more likely that hypotheses of the "keystone-species" type are applicable: a pollination community is stabilised by a few keystone species or "interaction nodes" in a plant-pollinator network (Jordano 1987; Corbet 2000b; Olesen & Jordano 2002; Bascompte *et al*. 2003; Jordano *et al*. 2003; Memmott *et al*. 2004), and the remaining species being more or less redundant. Plant species attracting many different pollinators, such as Apiaceae species, or insects visiting and pollinating many plant species, such as bumblebees and several hoverflies, may be such keystone species (Memmott 1999; Memmott *et al*. 2004). Finally, a part of the ideas fits into the "context" type of hypotheses: the effect of biodiversity depends on the context (habitat fragmentation, population size). When posing hypotheses or questions about the importance of biodiversity for pollination, it is important to realise that the outcome may differ between the ecological levels: the community or individual species. For example, the total pollinator diversity may be important for pollination at the plant community level, while plant species with one pollinator species only need that particular pollinator.

So, to judge upon the quality state of pollination systems, important questions are: (1) What is the minimum diversity of insects for the pollination of a plant community? (2) What is the minimum diversity of a plant community for sustaining all necessary pollinators? (3) Which type of plant species will suffer most from species loss?

Species decline in northwest Europe and in the Netherlands

The MA (UNEP 2005), and before that also the Global Biodiversity Assessment (UNEP 1995), have highlighted the alarming decline of biodiversity on earth. Firstly, the total biodiversity on the planet is declining due to increased extinction rates. Secondly, across a range of taxonomic groups, the population sizes, densities or total ranges of the majority of species are declining and within species the genetic diversity has declined. Thirdly, the distribution of species on earth is becoming more homogeneous. As the main cause the MA mentions a dramatic growth in the demand for food, water, timber, fibre and fuel. This is expressed in among others agricultural intensification, increased deforestation and increased use of fossil energy sources, leading to habitat fragmentation, pollution, dehydration, extremely high levels of nitrogen inputs and climate change (UNEP 2005). The worldwide decline of flowering plants and their pollinators is ascribed to similar and some other causes: habitat destruction and fragmentation, agriculture, pesticides, pollution, intensified mowing and grazing reducing the amount of flowers, introduction of foreign pollinators like the domesticated honeybee, and deforestation (Rathcke & Jules 1993; Buchmann & Nabhan 1996; Corbet 1997; Kearns & Inouye 1997; Fisher 1998; Kearns *et al*. 1998; Kwak *et al*. 1998; Cox & Elmqvist 2000; Kremen & Ricketts 2000; Cane 2001; Cane & Tepedino 2001; Spira 2001). These are global patterns: below I will focus on species decline and its causes in northwest Europe, particularly in the Netherlands.

In Europe, most ecosystems have undergone centuries of extensive farming practices, resulting in semi-natural and often very species-rich habitats (Bignal & McCracken 1996). During the second half of the 20th century, human population increased and technical innovations led to land use changes and agricultural intensification, resulting in the disappearance of nearly all of those traditional land use systems in Northwest Europe, including the Netherlands (Bignal & McCracken 1996; Geertsema 2002; Manhoudt & de Snoo 2003; Kleijn & Sutherland 2004; Spek 2004; Blomqvist 2005). This also resulted in species loss and decline, among others of plants (Plate *et al*. 1992; Quinn *et al*. 1994; Andreasen *et al*. 1996; Tamis *et al*. 2004), and of flower-visiting insect taxa, e.g. butterflies (Dover *et al*. 1990; Thomson 2001; van Swaay & Warren 2001; Stefanescu *et al*. 2004) and bees (Williams 1986; Rasmont 1988; Kwak *et al*. 1996; Westrich 1996; Benedek 1997; Peeters *et al*. 1999; Calabuig 2000).

In the Netherlands, species have declined among all analysed taxonomical groups in aquatic and terrestrial habitats (CBS *et al*. 2004). The main causes are a decline of the available habitat area and habitat quality due to eutrophication, dehydration by land draining, habitat fragmentation, and acidification. An example of the current red list status of some taxonomical groups is given in figure 1.1. Around a third of all vascular plants in the Netherlands are endangered or have gone extinct (Plate *et al*. 1992; Tamis *et al*. 2004). One observed trend is that common species become more common, and (naturally) rare species become rarer (Plate *et al*. 1992; Kwak 1994a). Furthermore, of many plant species population sizes and densities within populations are decreasing.

It is striking that two important flower-visiting insect taxa, butterflies and bees, appear to be among the most negatively affected groups (fig. 1.1): more than 50% of the species are either endangered or have disappeared. Only some aquatic arthropod groups and all fish, amphibians and reptiles are affected more strongly (not shown) (CBS *et al*. 2004). Butterflies are one of the most severely declining animal groups: recently it was observed that even common species have started to decline in abundance in the Netherlands (CBS *et al*. 2004). This was ascribed to a decline in the host plants of the larvae and wrong management such as too rigorous mowing (CBS *et al*. 2004), but also to continued pesticide use (Dover *et al*. 1990; Groenendijk *et al*. 2002). Also in the UK, butterflies appear to be one of the most vulnerable taxa (Thomas *et al*. 2004).

Among bees, the oligolectic solitary species, bumblebees and parasite bees have a higher percentage of red-listed species than polylectic solitary bees (fig. 1.2) (Peeters & Reemer 2003). Among bumblebees, the common species with intermediate and short probosces became more common, while species with longer probosces have declined (Kwak 1994a; Goulson *et al*. 2005). The latter are the more specialised bumblebee species, foraging on plant species with flowers with long corollas, particularly Fabaceae. The availability of flowers is probably too low in intensified agricultural landscapes (Goulson *et al*. 2005). In general, the decline of bees is due to a decrease of available nesting sites and materials, food plants of specialised species, or a too large distance between nesting sites and food sources (Westrich 1996; Kearns & Inouye 1997; Calabuig 2000; Peeters & Reemer 2003).

Of other flower-visiting insect taxa there are too few data, especially from the past, for determining reliable Red Lists. Of aculeate wasps around 40% have declined or disappeared (fig. 1.1; Peeters *et al*. 2004). Hoverflies (N= 308 species) seem to have remained stable, but data are still being gathered and analysed (Achterkamp *et al*. 1998).

Habitat fragments in agricultural landscapes: road verges and ditch banks

In this thesis processes will be studied in habitats in agricultural landscapes, the dominant landscape type of northwest Europe and the Netherlands. In these landscapes, habitat

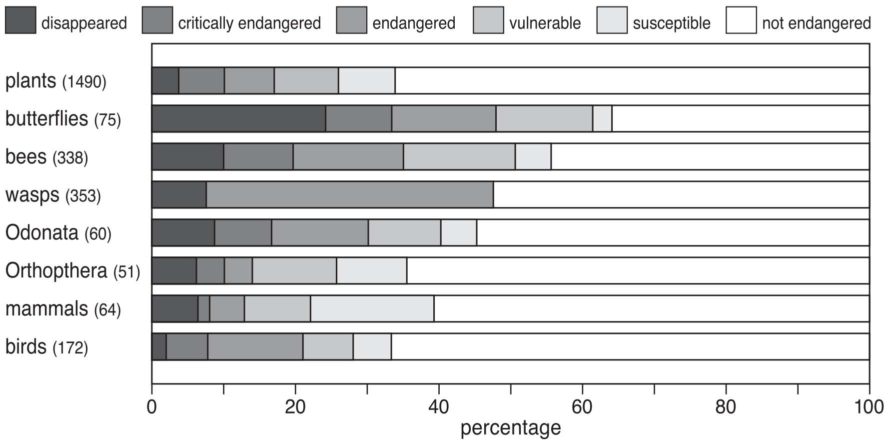


Fig. 1.1. Red-Listed status of eight taxonomical groups in the Netherlands (CBS *et al*. 2004). For wasps (Aculeata, including Sphecidae) a Red List does not exist, and the data presented are based on trends as mentioned by Peeters *et al*. (2004). For wasps the category "endangered" are species that have declined, and "not endangered" are species that remained stable or have increased. Odonata (dragonflies and damselflies), Orthoptera (grasshoppers and crickets), mammals and birds do not visit flowers in the Netherlands. The number of species per taxonomical group is between brackets.

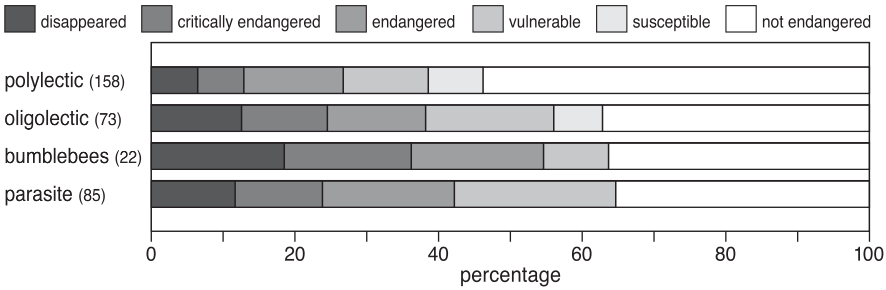


Fig. 1.2. Red-Listed status of the four ecological groups of bees (Apidae s.l., excluding Sphecidae) in the Netherlands (Peeters & Reemer 2003): polylectic solitary bees, oligolectic solitary bees, bumblebees and parasite bees (solitary bees and bumblebees together). The honeybee (*Apis mellifera*) is a domesticated species in the Netherlands and therefore not in the Red List of endangered wild species. The number of species per ecological group is between brackets.

loss and fragmentation are two important causes of species decline. These two are often confused by researchers analysing the effect of fragmentation, and may thus lead to wrong conclusions (Fahrig 2003). Still, the result of both processes is small and isolated, mostly linear habitat remnants. In agricultural landscapes these consist of hedges and shrubbery (Bignal & McCracken 1996; Saville *et al*. 1997; Salveter 1998a; Bäckman & Tiainen 2002; Le Cœur *et al*. 2002; Dauber *et al*. 2003; Spek 2004), field margins (Dover *et al*. 1990; Kleijn *et al*. 1997; Geertsema 1999; Sutherland *et al*. 2001; Tamis *et al*. 2001; Le Cœur *et al*. 2002; Manhoudt & de Snoo 2003), ditches and ditch banks (Geertsema

1999; Geertsema & Sprangers 2002; Blomqvist *et al*. 2003a; Blomqvist *et al*. 2003b) or road verges (Schaffers 2000; Raemakers *et al*. 2001). These habitat fragments are often highly dynamic as they are managed in various ways like mowing, coppicing, sod cutting, digging and draining.

Due to repeated reallocations and field size increases, the habitat heterogeneity and continuity traditionally present in agricultural landscapes has been reduced (Kleijn *et al*. 1997; Holland & Fahrig 2000; Jacquemyn *et al*. 2002; Benton *et al*. 2003; Manhoudt & de Snoo 2003). Fragmented and isolated plant populations can suffer from pollination deficiency and reduced seed set (Jennersten 1988; Rathcke & Jules 1993; Kwak *et al*. 1998; Steffan-Dewenter & Tscharntke 1999; Mustajärvi *et al*. 2001; Murren 2002; Soons 2003), and from genetic factors such as inbreeding and genetic drift (van Treuren *et al*. 1993; Oostermeijer *et al*. 1994, 2000; Westerbergh & Saura 1994; Kwak *et al*. 1998; Velterop 2000; Luijten 2001; Mustajärvi *et al*. 2001; Wolf & Harridson 2001). For flower-visiting insects, food and nesting sites have disappeared (Osborne & Corbet 1994; Westrich 1996; Calabuig 2000; Bäckman & Tiainen 2002), and they may also suffer from inbreeding, for example bumblebees (Gerloff *et al*. 2003).

The importance of habitat remnants in intensified agricultural landscapes is increasingly being recognised by policy makers, such as the European Committee and the EU member states (Kleijn *et al*. 1999; Manhoudt & de Snoo 2003; Kleijn & Sutherland 2004). These habitats are important in preserving biodiversity outside natural reserves, and may be stepping stones between reserves. Plants may also profit from (flower-rich) linear habitats, as they are used by insects as bridges between isolated flower patches, in this way enabling pollen and gene flow (Verlaar 1990; Widén & Widén 1990; Kwak *et al*. 1998; Kwak & Vervoort 2000; Velterop 2000; Schulke & Waser 2001). In the Netherlands, road verges, ditches and field margins are the main habitat remnants. For example, road side habitats cover up to 2.1% of the total land area; compared to the 4.2% of natural areas this is a considerable amount (Schaffers 2000).

Flora and fauna in road verges, ditch banks and field sites are affected directly through mowing and other management, and indirectly from the surrounding matrix through fertiliser input and spraying of pesticides (Kleijn *et al*. 1997; Schaffers *et al*. 1998; Schaffers 2000). Frequent mowing will reduce the number of flowers containing nectar and pollen, resulting in a change of the behaviour of insects, or will contribute to their disappearance (Fussell & Corbet 1992). When sites are in a matrix of continuous habitat, there will be more plant and insect species than at isolated sites (Rathcke & Jules 1993). Crop monocultures reduce total floral diversity in a landscape, and subsequently reduce pollinator diversity in adjacent habitat fragments (Kearns *et al*. 1998).

Research area

The research area is in the north of the province of Drenthe in the Netherlands, about 53° N and 6° E (fig. 3.4). Most of the research activities and experiments for this thesis are performed in road verges and ditch banks that are situated in matrices with various types of land use. The road verges are along small (local) roads: these are most directly connected to adjacent matrix, the majority of road verges in agricultural landscapes are along small roads, larger provincial and national roads have a different management policy, and smaller roads are less dangerous for the researcher. Advantages of road verges and ditch banks are that they are similar in size, and are mostly publicly accessible, so no permissions are needed.

The greater part of the area is used for agricultural purposes and has undergone several rounds of land reallocations between the 1950s and 1990s (Geertsema 2002). Part of the area is in or adjacent to natural reserves of semi-natural hay meadows along the stream of the Drentse Aa. Land use varies between heavily fertilised and sprayed intensive grassland and arable land (potatoes, cereals, maize, sugar beet), intensively or extensively grazed meadows (cattle, sheep and horses), and semi-natural hay meadows mown once or twice a year (mostly in natural reserves). In the intensively used fields and meadows, hardly any plant species with flowers interesting for flower-visiting insects are growing. The only crops needing insect pollination are North-American high-bush blueberries (*Vaccinium* spec.) and ornamental poppies (*Papaver somniferum*), but they were grown in a very limited number of fields. In some grasslands and the less intensively grazed meadows, plant species interesting for insects are present in reasonable numbers, for example *Trifolium repens, Taraxacum species*, and *Hypochaeris radicata*. The semi-natural hay meadows (*Calthion palustre*) are rich in flowering plants, like *Ranunculus repens, R. acris, Cirsium palustre, Filipendula ulmaria, Eupatorium cannabinum* and *Iris pseudacorus*, including rare or Red-Listed species, such as *Rhinanthus angustifolius, R. minor, Phyteuma spicatum* subsp. *nigrum* and orchid species (Bakker & Olff 1992; van Duren *et al*. 1997; van Duren & van Andel 1997; Grootjans *et al*. 2002).

Aims and outline of the thesis

In this thesis, biodiversity and its role for pollination are investigated in various ways. Community diversity is the local species richness and abundance of flowering plants and flower-visiting insects in the road verges and ditch banks. Functional diversity is the number of different pollination syndromes or systems, reflected as guilds of plants with similar flower morphologies and visitor compositions, and guilds of insects with similar physical morphologies and plant preferences. The relation between the landscape (habitat continuity or fragmentation and land use intensity) and community and functional diversity are investigated, and the possible effects on visitation and pollination studied. The main question of this thesis is:
Is a high biodiversity important for the pollination of entomophilous wild plant species in agricultural, fragmented landscapes?

The various processes that are thought to be important concerning the role of biodiversity for pollination are depicted in a flow diagram (fig. 1.3). The landscape, i.e. the matrix of habitats with the various types of land use, affects the plant and insect communities (species richness, abundances of flowers and insect individuals, and functional and species compositions). The plant community composition and diversity will affect the insect visitor diversity and composition. Both plant and insect diversity will affect the individual behaviour of insects. For example, flower constancy depends on the combination of plant species flowering simultaneously in a vegetation (competition or facilitation between plants). Similarly, the number of visits by insects that flowers receive per unit time (visitation rate) and the frequencies of visits by different insect species also depend on the composition and diversity of a flowering plant community, but also on the insect community composition and diversity. Individual behaviour and visitation determine the pollination success of a plant species. The quantity and quality of pollination (the amount and purity of deposited pollen) will affect seed set, a measure of the success of pollination. How finally seed set affects plant species numbers, and thus community composition, is out of the scope of this study.

The complete sequence from insect visitation and behaviour, via actual pollination until seed set and germination are studied in this thesis. Both field surveys (descriptive analyses) and experiments (garden and field) are used. The field surveys are used to quantify the diversity of flowering plants and their flower visitors, and include investigations of land use in the matrix, and diversity of flowering plants and insects in the sites (road verges and ditch banks). The resulting data set is used in chapters 2 - 5 and 8. Experiments with potted plants of a number of selected species are used in chapters 5, 6 and 7. By using potted plants, the number and arrangement of flowers can be controlled, and the plants can be placed into various environments. The effects on flower-visiting insect diversity, visitation and pollination success of the target plant species are evaluated in terms of pollination (deposition and purity) and plant fecundity (realised seed set).

In chapter 2 the functional diversity of the plant and insect species is described: what types of pollination systems or syndromes can be found, and what are the degrees of specialisation of the plant species? The goals are to determine classes of plant species with similar frequencies of flower-visiting insect taxa using a cluster analysis, and to investigate how existing classifications are reflected in the clusters.

In chapter 3 the diversity of flowering plants and flower-visiting insects in the research area is quantified. The main questions are: how are diversity and abundances of plants and insects influenced by land use, agricultural intensity and site management? In which way are these interacting communities related with each other?

In chapter 4 the effects of biodiversity on the frequency of interactions between flowering plants and flower visiting insects are described in a foodweb context. The main questions are: What is the effect of species richness and abundance on the number of insect species that visit a plant species, and on the number of plant species an insect species visits? Do these effects differ between functional groups of plants and insects?

In chapter 5 questions concerning plant-plant interactions are addressed: what is the effect of a plant species' neighbours on its visitation and pollination, and does the type of neighbourhood matter? Both descriptive and experimental methods are used. The effects of flowering plant species richness and community composition on the number of visiting insect species, visitation rate and pollen deposition are analysed.

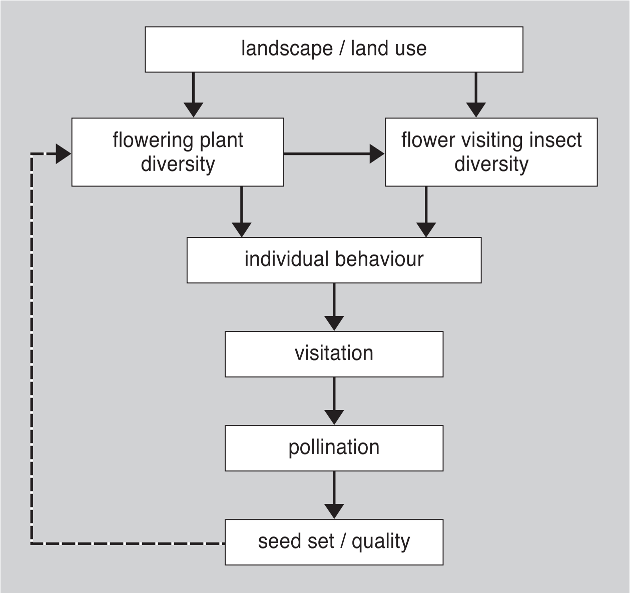


Fig. 1.3. Flow diagram depicting the various processes that are thought to be important concerning the role of biodiversity for pollination. The arrow from seed set quality to flowering plant diversity is dotted because this part is out of the scope of this thesis. For explanantions see text.

In chapter 6 the vulnerability of plant species with various pollination syndromes and degrees of specialisation to pollinator species loss is analysed. A field experiment is conducted with six plant species. The following questions are asked: 1. How important is insect pollination compared to self-pollination for the reproduction of the six target plant species? 2. What are the effects of different environments on the insect visitor diversity, visitation rate and resulting seed set of the target plant species? 3. Do the target plant species have alternative pollinators in the different environments?

In chapter 7 the role of biodiversity for pollination is combined with plant population size for a single plant species. The effects of plant and insect species richness and population size on the individual behaviour of insect visitors, insect visitation, pollen deposition and purity of deposited pollen are analysed for *Succisa pratensis*.

In chapter 8 I analysed whether most plant species in a community are visited by a small set of insect species using the data set from chapters 2 - 4. Three questions are discussed: Firstly, are the most frequent and abundant insect species enough for a sustainable pollination of the entire plant community? Secondly, if not, what other insect species are needed? Thirdly, what are the habitat requirements of the (minimal) pollinator diversity?

2

Classification of pollination systems using insect visitor frequencies

Frank Hoffmann, Manja M. Kwak and Jelte van Andel

SUMMARY

Pollination syndromes are the traditional classification system of biotic pollination, but the use and application of pollination syndromes was criticised in literature: syndromes are often applied without field data, and syndromes are said to indicate that pollination interactions of plants and animals are specialised, while the majority are generalised. In this paper insect visitation data of plant species within a plant community in the Netherlands were analysed. The goals were (1) to determine classes of plant species with similar frequencies of flower-visiting insect taxa, and (2) to investigate how existing pollination classifications are reflected in those classes. Insect frequency data were gathered in road verges and ditch banks in 2000 and 2001. The insect species were subdivided into 12 differing functional groups. Cluster analysis with the relative frequencies of the insect groups per plant species was used. Of the 99 plant species observed, only plant species that were visited by at least 20 insect individuals were used (N=58). Fourteen clusters were identified. The proportion of flies, syrphids and solitary bees on the one hand, and the proportion of bumblebees, *Rhingia campestris* and honeybees on the other were the main characteristic groups for the two major cluster branches. Other taxa were very infrequent. The accessibility of nectar and pollen was the most important feature that determined the differences of proportions of the visitor groups between the clusters. The most specialised plant species were visited by 90-120 insect species in total or 8 - 13 insect species per census. The least specialised plant species were visited by 2- 4 (total) or 1 - 2 (per census) insect species. Two thirds of the plant species can be regarded as generalised, one third as specialised. Two of the known pollination syndromes were applicable for <50% of the plant species: the syndrome of flies and the syndrome of bees. For >50% of the plant species, syndromes still have to be defined. The classic pollination syndromes and earlier works are a profound basis for a classification, but need extension and refining based on field data.

INTRODUCTION

The classification of biotic pollination systems has been a subject of study and debate since the 19th century (Loew 1895, Fægri & van der Pijl 1979; Armbuster *et al*. 2000; Olesen 2000; Ollerton & Watts 2000). The traditional classification system is that of the pollination syndromes as described by Fægri & van der Pijl (1979). A pollination syndrome consists of morphological blossom characteristics in relation to a specific mode of pollination. Pollination syndromes are named after the most typical pollinators (table 2.1). Another classification of plants and flower visitors by Loew (1895) as described by Fægri & van der Pijl (1979) and Ellis & Ellis-Adam (1993) is based on adaptation of insects to flower visitation and accessibility of flowers for insects: insects and plants can be subdivided into allotropous, hemitropous and eutropous (see table 2.2 for definitions).

In recent years, the use and application of pollination syndromes was criticised: according to Johnson & Steiner (2000) and Ollerton & Watts (2000), plants were often categorised according to their perceived syndrome, but mostly in absence of actual data of flower visitation or pollination by animals. Johnson & Steiner (2000) added that syndromes were originally meant as a description of convergent evolution, and not as a typology of flowering plants to be used without any field data. Fægri & van der Pijl (1979) themselves warned that the names of syndromes are based on a typical pollinator, and should not be taken literally: "bees" may include long-tongued flies, and some solitary bees may be classified as "beetles", depending on their morphology and behaviour.

Some authors (Jordano 1987; Ellis & Ellis-Adam 1993; Waser *et al*. 1996; Memmott 1999) state that syndromes imply that all pollination systems are more or less specialised, whereas the majority of plant-pollinator interactions is generalised, and syndromes only hold for a minority of plant species. The disagreement about specialisation and generalisation may be caused by confusing evolutionary specialisation with ecological specialisation (Armbuster *et al*. 2000). Evolutionary specialisation is a process with a direction, i.e. from many to fewer pollinating taxa. The ecological specialisation of a plant species is a state, referring to having few pollinators relative to other plant species. The dichotomy of specialisation vs. generalisation is an oversimplification of reality that actually is a continuum (Waser *et al*. 1996; Armbuster *et al*. 2000; Johnson & Steiner 2000). Furthermore, many species may show specialised flower traits, but are paradoxically visited by a large number of species. It may be that only a small proportion of the visitors are actual pollinators, functioning as a selective force (Johnson & Steiner 2000; Ollerton & Watts 2000). Another explanation may be that flowers can also be adapted to relatively less effective pollinators when this adaptation causes little loss in the fitness contribution of a more effective pollinator (Aigner 2001).

The classification of biotic pollination systems is further complicated because pollination involves two parties, i.e. the plant and the pollinator (Fægri & van der Pijl 1979). Although the two parties may appear to be mutualistic partners, their interests are not equal (Westerkamp 1997): plants "want" to be pollinated at the lowest cost, whereas the flower visitor will go for the highest gain. Furthermore, the distribution of interactions between plants and flower visitors is highly asymmetrical: many specialised plant species

Table. 2.1. Pollination syndromes as described by Fægri & van der Pijl (1979). Syndromes are named after their main pollinator taxon. They are considered to be "typical" representatives of the taxa: e.g. "bees" can include bumblebees and beeflies. Only insect syndromes are considered, because vertebrate pollinators do not occur in central and northern Europe. Flowers belonging to the syndrome of carrion flies (*sapromyophily*) did not occur in the research area.

syndrome	flower morphology	colour	odour	primary attractants	sexual organ	remarks
beetles *canterophily*	actinomorphic, no special shape, large, shallow, often bowl shaped blossoms	dull nectar guides absent	strong fruity	mostly pollen, sometimes nectar: open, easily accessible	exposed	
flies *myophily*	actinomorphic, regular, simple, no depth effect	light or dull, whitish nectar guides frequently present	imperceptible	nectar: open, easily obtainable	exposed	
bees *melittophily*	zygomorphic, with great depth effect, mechanically strong, adequate landing facilities	lively, yellow or blue nectar guides present	weak , fresh	nectar: hidden, deep but less deep than moth flowers, abundant. pollen: open, (pollen source for bees often from simple flowers)	concealed	Syndrome of bumblebees, honeybees and larger (tropical) solitary bees. Most solitary bees in temperate Europe are "primitive" and comparable to e.g. beetles.
wasps (Vespidae)	not described	dull, brown	not described	nectar: accessible	exposed	Not considerd as a real syndrome
butterflies *psychophily*	actinomorphic, erect, radial, good landing facilities	vivid, including red simple nectar guides	weak	nectar: in ample quantities, hidden in tubes or spurs	exposed	
moths *phalaenophily*	actinomorphic or zygomorphic, horizontal	white or faint nectar guides absent	strong, nocturnal	nectar: in large quantities, deeply hidden in tubes or spurs	exposed or concealed	nocturnal anthesis

Table. 2.2. Categorisation of flower visitors (insects) based on adaptation of insects to flower visitation and flowering plants based on accessibility of flowers for insects, sensu Loew (1895), as described by Fægri & van der Pijl (1979) and Ellis & Ellis-Adam (1993). For plants sometimes the suffix "-philous" instead of "-tropous" is used.

category	insects	plants
allotropous	hardly adapted to flower visitation, short proboscis, diet includes other food sources than flower products	easily accessible nectar, can be used by not adapted, short-tongued visitors
hemitropous	intermediate adaptation to flower visitation, intermediate proboscis	partly concealed nectar, can be visited by intermediately adapted visitors
eutropous	complete adaptation to flower visitation, intermediate to long proboscis, often high levels of specialisation	concealed nectar, can only be visited by highly adapted visitors

are visited by generalised insects, whereas many specialised insects visit generalised plants (Jordano 1987; Bronstein 1995; Waser *et al*. 1996; Memmott 1999; Olesen & Jordano 2002). The two parties are also reflected in the disciplinary specialisation of pollination ecologists (Waser 2001): a botanical background and therefore focus can lead to a classification system different from a zoological or entomological oriented system. For example, the classification of allo-, hemi- and eutropous species has an entomological origin.

Pollination syndromes, provided they are defined broadly enough, are an useful ecological classification system, but should be supported by field data (Armbuster *et al*. 2000; Ollerton & Watts 2000). A first approach can be investigations of flower visitor assemblages of many different plant species within or across communities (Ellis & Ellis-Adam 1993; Waser *et al*. 1996; Armbuster *et al*. 2000; Johnson & Steiner 2000; Ollerton & Watts 2000). So far, only few examples with larger data sets exist (Ellis & Ellis-Adam 1993; Memmott 1999; Corbet 2000b; Olesen & Jordano 2002), and they mostly find that the classifications poorly fit to the data sets.

In this paper we will analyse insect visitation data of plant species typical of grasslands and road verges in the Netherlands. Our goals are (1) to determine classes of plant species with similar frequencies of flower-visiting insect taxa using a cluster analysis, and (2) to investigate how the two existing classifications (tables 1 and 2) are reflected in the cluster analysis. As our study area is in temperate Europe, we expect to find intermediate to high levels of generalisation (Memmott 1999; Olesen & Jordano 2002), and a majority of Diptera and Apidae as flower visitors (Ellis & Ellis-Adam 1993; Memmott 1999; Elberling & Olesen 1999; Totland *et al*. 2000).

MATERIAL AND METHODS

Research area

We conducted our study in road verges and ditch banks. In agricultural landscapes these linear sites are often the only natural habitats. In the Netherlands, roadside habitats cover up to 2.1 % of the total land area, a considerable amount compared to the 4.2 % of natural areas (Schaffers 2000). The study area was situated in the north of the Netherlands in the province of Drenthe (53º00' N, 6º35' E), an area of Pleistocene origin with (loamy) sand or peat on sand soils. In 2000, we inspected ca. 325 km of road verges and ditch banks to select 51 linear sites of 100 m length and 1-2 m width. The sites were not shaded. Verges along large roads with heavy traffic were avoided for safety reasons.

The sites were situated in landscapes with different land use, varying from intensive agriculture (crop fields and sown grasslands), grazing meadows (cattle, sheep and horses) or semi-natural natural reserves. Sites were mown between zero and five times per observation period.

Transect observations

We did transect observations of insects on flowering plants, as this would provide us with data from many sites distributed over a larger area and over a whole flowering season. Furthermore, the behaviour of insects is influenced by the plant community composition: differences in flower abundance between plant species affect foraging decisions of flower visitors (Thomson 1978; Fægri & van der Pijl 1979; Waser 1979; Feinsinger 1987; Armbuster *et al*. 2000). We therefore measured many sites in order to prevent local effects on visitor compositions.

Within each site, we counted the number of flowering units of all occurring entomophilous plants (no graminoids or other wind-pollinated plant species). Woody plants, except *Rubus* species, were not included. Flowering units were single flowers, umbels, heads and spikes, depending on the species. All insects visiting inflorescences and larger than 3 mm were counted by walking slowly along a transect once. Insects were identified in the field to species or morphotype, henceforward "species" indicates both. Whenever possible or necessary, samples of insects were taken to be identified later. Catching all insects would have disturbed the observations and is very time consuming. The observation time of insects was usually between 11:00 and 16:00 h local time. We started and/or stopped earlier or later when forced by circumstances like extreme temperatures, light period, or abrupt weather change. The weather circumstances were as constant as possible, i.e. wind speeds less than 5 Beaufort, and no precipitation. In 2000, all sites were visited approximately every two weeks from the beginning of May until the beginning of October, 10 rounds in total. In 2001, the sites were investigated again from the beginning of May until the end of August (7 rounds: in the first two rounds all sites were investigated, in the other five only 19 sites). The total number of censuses was 537, a census is an observation at a certain site on a certain day. The number of censuses is not equal to the number of sites times the number of rounds, because during several censuses plants were either not flowering due to phenological reasons or mowing, or plants were not visited by insects.

Plant linkage level

Plant linkage level (L_p) is the number of animal species in a network a plant species interacts with. It is used as a measure of generalisation or specialisation of a species or community (Olesen *et al*. 2002; Olesen & Jordano 2002; Dupont *et al*. 2003). We calculated total L_p, i.e. the total number of insect species a plant species was visited by for the whole research period, and mean L_p, i.e. the mean number of insect species a plant species was visited by per census. We calculated these two values, because the total L_p of a species may also be influenced by the number of sites a plant species was growing, and by the duration of the flowering period. Species that have a long flowering period have a higher chance to be visited by insect species with various phenologies compared to plants with a short flowering period.

Insect groups

The insects were grouped into twelve groups for analysis. Had the data been analysed with insect species, the results would mainly reflect phenological effects: in the different seasons, various insect species were present. As we did not measure any morphological features of the insects, the subdivision was mainly based on taxonomy. The groups were: flies (non-syrphid Diptera), syrphids (Syrphidae except *Rhingia campestris*), *Rhingia* (the syrphid fly *Rhingia campestris*), beetles (Coleoptera), butterflies, moths, ants, wasps (Symphita, Parasitica, Aculeata), solitary bees, bumblebees, honeybee (*Apis mellifera*), and other (mainly Hemiptera). The syrphid fly *Rhingia campestris* has a much longer proboscis than all other syrphid flies and can therefore also visit flowers with long tubes. During the analysis it appeared that it was necessary to separate *Rhingia* from the other syrphids (see discussion). "Wasps" includes all Hymenoptera that cannot be classified as "bee" or "ant". The bees were divided into three groups: bumblebees and honeybees differ from solitary bees because they are social, and in northwest Europe including the Netherlands the honeybee is an exclusively domesticated species (Ellis & Ellis-Adam 1993; Peeters *et al*. 1999).

Flower types

We did not measure any morphological features of the plants and therefore subdivided our plant species into nine flower types. We based our flower types on the six "structural blossom classes" (dish-bowl, bell-funnel, head-brush, gullet, flag and tube) as described by Fægri & van der Pijl (1979). In total we had nine flower types: umbel, bowl, head, umbel-head, brush, bell, tube, gullet, and flag, that were defined as follows:

1. **Umbel**: inflorescence composed of small dish-flowers, in our study all these plant species belong to the Apiaceae. Fægri & van der Pijl (1979) described dish flowers as flat, with open nectaries and reproductive organs in the center of the flower, mostly arranged in compound units. Umbels can have a visitor composition that differs significantly from other dish-bowl like flowers (Ellis & Ellis-Adam 1993). Therefore we chose to group these into a different category than bowl flowers.
2. **Bowl**: larger, dish- or bowl-shaped flowers with open nectaries and reproductive organs in the center of the flower. These are species with only a single or few flowers in loose

inflorescences (e.g. *Ranunculus, Rubus* and *Veronica* species).

3. **Head**: flat or globular inflorescences composed of tightly arranged small, tubular flowers of Asteraceae and Dipsacaceae species (e.g. *Hypochaeris radicata, Cirsium arvense* and *Succisa pratensis*).
4. **Umbel-head**: Asteraceae species with inflorescences that are composed of small and very flat flower heads that are arranged in "umbels" (e.g. *Tanacetum vulgare* and *Achillea millefolia*), and therefore stand between head and umbel.
5. **Brush**: loosely composed inflorescences with irregular shapes. This is a rather mixed group of plant species, e.g. *Valeriana officinalis, Mentha aquatica* and *Eupatorium cannabinum*.
6. **Bell**: down-wards oriented flowers with a wide opening, and a typical "bell-shape", only one species: *Campanula rotundifolia.*
7. **Tube**: actinomorph flowers with long corollas (e.g. *Silene dioica* and *Symphytum officinale*).
8. **Gullet**: zygomorph, "gullet-shaped" narrow flowers with long corollas that are either open (e.g. *Lamium album* and *Scrophularia nodosa*) or have to be forced open (e.g. *Linaria vulgaris*).
9. **Flag**: zygomorph, closed flowers of the Fabaceae (e.g. *Trifolium pratense* and *Lupinus polyphyllos*).

Statistics

For analysis only plant species were used with a minimum of insect visitors of 20 individuals in total for all sites and years combined. This is an arbitrarily chosen number which we found safe enough for analysis. For these plant species relative visitor numbers (percentages) for the insect groups were calculated. Then a distance matrix with Euclidian distances was calculated, which was used for cluster analysis with complete linkage as clustering procedure. The amalgamation schedule (line graph of the linkage distances at successive clustering steps) was used to determine the cut-off point for defining separate clusters. The line levelled off at a "linkage distance" of 40, which we then used as the cut-off point. The analyses were done with STATISTICA version 6.1 (StatSoft, Inc., 2003).

RESULTS

Of the 99 plant species observed, 58 plant species were visited by at least 20 insect individuals (table 2.3). Fourteen clusters were identified (fig. 2.1), that split into two main cluster branches, i.e. branch A (cluster 1-8) and branch B (cluster 9-14). The flower types of the plants are not equally distributed over the clusters (table 2.3, fig. 2.2). The flower types umbel, umbel-head, bowl and bell are all in branch A. Umbelliferous plant species (Apiaceae) are exclusively in clusters 1 and 2. Bowl flowers are distributed over clusters 1-7, and the flower type bell (only one species, *Campanula rotundifolia*) is in a single cluster (8). The flower types head and brush are in both branches, but mainly in branch A.

Table. 2.3. Plant species data. The numbers in the column "cluster" correspond to the numbers of the clusters in fig. 1 and 2. Linkage level (L_p) is the number of insect species a plant species was visited by at a certain place and time.

cluster	Plant species	short	Plant family	Flower type	morph[1]	orientation	N sites[2]	N censuses[3]	N ind.[4]	Tot. L_p[5]	Mean L_p ±SE[6]
1	*Potentilla reptans*	poterep	Rosaceae	bowl	a	up	2	3	65	5	2.0 ±0.6
	Potentilla erecta	poteere	Rosaceae	bowl	a	up	15	45	673	36	1.9 ±0.2
	Bellis perennis	belliper	Asteraceae	head	a	up	5	11	49	8	1.9 ±0.5
	Heracleum sphondylium	herasph	Apiaceae	umbel	a	up	29	90	8741	122	8.7 ±0.6
	Anthriscus sylvestris	anthsyl	Apiaceae	umbel	a	up	45	124	4763	123	8.9 ±0.5
2	*Aegopodium podagraria*	aegopod	Apiaceae	umbel	a	up	11	30	821	92	9.1 ±1.0
	Angelica sylvestris	angesyl	Apiaceae	umbel	a	up	15	38	2099	107	12.7 ±1.2
	Cirsium arvense	cirsarv	Asteraceae	head	a	up	20	56	1151	64	4.1 ±0.6
	Taraxacum cf. officinale	taraoff	Asteraceae	head	a	up	42	73	490	60	2.7 ±0.3
	Crepis capillaris	crepcap	Asteraceae	head	a	up	14	47	902	49	3.7 ±0.5
	Leontodon autumnalis	leonaut	Asteraceae	head	a	up	22	53	645	51	3.5 ±0.5
	Ranunculus acris	ranuacr	Ranunculaceae	bowl	a	up	26	76	443	66	2.6 ±0.3
	Hieracium aurantiacum	hieraur	Asteraceae	head	a	up	1	4	32	7	2.8 ±0.9
	Cardamine pratensis	cardpra	Brassicaceae	bowl	a	up	13	22	123	24	2.2 ±0.4
	Veronica chamaedrys	verocha	Scrophulariaceae	bowl	a	up	9	14	71	22	2.4 ±0.4
3	*Ranunculus repens*	ranurep	Ranunculaceae	bowl	a	up	32	93	897	78	3.4 ±0.3
	Filipendula ulmaria	filiulm	Rosaceae	brush	a	up	8	31	212	26	2.8 ±0.4
	Matricaria spec	matrspe	Asteraceae	head	a	up	16	39	234	34	2.7 ±0.3
	Hypochaeris radicata	hyporad	Asteraceae	head	a	up	34	132	1778	95	4.4 ±0.3
	Hieracium umbellatum	hierumb	Asteraceae	head	a	up	11	27	263	41	3.4 ±0.7
	Hieracium laevigatum	hierlae	Asteraceae	head	a	up	30	123	1640	107	4.9 ±0.4
	Eupatorium cannabinum	eupacan	Asteraceae	brush	a	up	7	26	987	60	9.3 ±1.0
	Achillea millefolium	achimil	Asteraceae	umbel-head	a	up	21	73	525	70	2.9 ±0.4
4	*Tanacetum vulgare*	tanavul	Asteraceae	umbel-head	a	up	19	52	1855	85	7.8 ±0.8
	Rubus cf. fruticosus	rubufru	Rosaceae	bowl	a	up	18	44	488	71	4.7 ±0.5
	Chamerion angustifolium	chamang	Onagraceae	brush	a	side	18	57	441	45	3.3 ±0.4
5	*Rorippa amphibia*	roriamp	Brassicaceae	bowl	a	up	4	10	34	18	2.6 ±0.9
	Mentha aquatica	mentaqu	Lamiaceae	brush	a	up	7	24	422	49	6.0 ±1.1
	Valeriana officinalis	valeoff	Valerianaceae	brush	a	up	12	42	523	61	5.3 ±0.6
	Jasione montana	jasimon	Campanulaceae	brush	a	up	3	15	115	41	4.7 ±1.0
	Hypericum perforatum	hypeper	Clusiaceae	bowl	a	up	14	40	260	30	2.9 ±0.4

cluster	Plant species	short	Plant family	Flower type	morph[1]	orientation	N sites[2]	N censuses[3]	N ind.[4]	Tot. L_p[5]	Mean L_p ±SE[6]
6	*Succisa pratensis*	succpra	Dipsacaceae	head	a	up	5	11	178	13	3.0 ±0.7
	Sonchus arvensis	soncarv	Asteraceae	head	a	up	3	6	79	26	5.2 ±1.5
	Rorippa nasturtium-aquaticum	rorinas	Brassicaceae	bowl	a	up	3	5	58	17	4.4 ±1.4
7	*Senecio jacobaea*	senejac	Asteraceae	umbel-head	a	up	5	10	52	18	3.0 ±1.0
	Hieracium pilosella	hierpilo	Asteraceae	head	a	up	8	22	186	48	4.5 ±0.9
	Lysimachia vulgaris	lysivul	Primulaceae	bowl	a	up	11	28	86	14	1.8 ±0.2
8	*Campanula rotundifolia*	camprot	Campanulaceae	bell	a	down	6	12	71	12	2.0 ±0.4
9	*Calluna vulgaris*	calluvul	Ericaceae	brush	a	side	5	10	735	24	3.6 ±1.6
10	*Silene dioica*	siledio	Caryophyllaceae	tube	a	up/ side	10	20	58	15	1.7 ±0.4
	Phyteuma spicatum ssp. *nigrum*	phytspi	Campanulaceae	brush	a	side	1	3	41	7	3.3 ±0.7
	Ajuga reptans	ajugrep	Lamiaceae	gullet	z	side	3	5	57	2	1.6 ±0.2
11	*Lycopus europaeus*	lycoeur	Lamiaceae	brush	a	side	3	6	26	11	2.5 ±0.7
	Cirsium palustre	cirspal	Asteraceae	head	a	up	6	19	46	25	2.1 ±0.4
12	*Lythrum salicaria*	lythsal	Lythraceae	brush	a	side	15	50	417	46	3.8 ±0.4
13	*Lychnis flos-cuculi*	lychflo	Caryophyllaceae	tube	a	up/ side	6	14	50	14	1.9 ±0.4
	Glechoma hederacea	gleched	Lamiaceae	gullet	z	side	16	32	85	15	1.6 ±0.2
14	*Lamium album*	lamialb	Lamiaceae	gullet	z	side	8	26	82	12	1.8 ±0.2
	Linaria vulgaris	linavul	Scrophulariaceae	gullet	z	side	8	13	59	7	2.2 ±0.3
	Trifolium repens	trifrep	Fabaceae	flag	z	side	25	50	110	10	1.4 ±0.1
	Lotus corniculatus	lotucor	Fabaceae	flag	z	side	18	33	74	7	1.4 ±0.1
	Trifolium pratense	trifpra	Fabaceae	flag	z	side	18	46	119	14	1.6 ±0.1
	Galeopsis tetrahit	galetet	Lamiaceae	gullet	z	side	14	24	42	11	1.4 ±0.1
	Lupinus polyphyllus	lupipoly	Fabaceae	flag	z	side	2	4	21	5	1.8 ±0.3
	Symphytum officinale	sympoff	Boraginaceae	tube	a	down/ side	14	68	376	22	1.9 ±0.2
	Vicia sativa	vicisat	Fabaceae	flag	z	side	1	8	38	4	1.6 ±0.3
	Vicia cracca	vicicra	Fabaceae	flag	z	side	7	13	61	5	1.1 ±0.1
	Rhinanthus angustifolius	rhinang	Scrophulariaceae	gullet	z	side	3	10	51	6	2.0 ±0.3

[1] Morph: a= actinomorph, z= zygomorph. [2] The number of sites in which a plant species was flowering and visited by insects in 2000 and 2001. [3] The number of censuses (for all sites combined, at many sites a plant was flowering during several censuses). [4] The total number of insects observed on flowers of a species during the whole research period. [5] The total number of insect species a plant species was visited by during the whole research period. [6] The mean number of insect species per census ±SE.

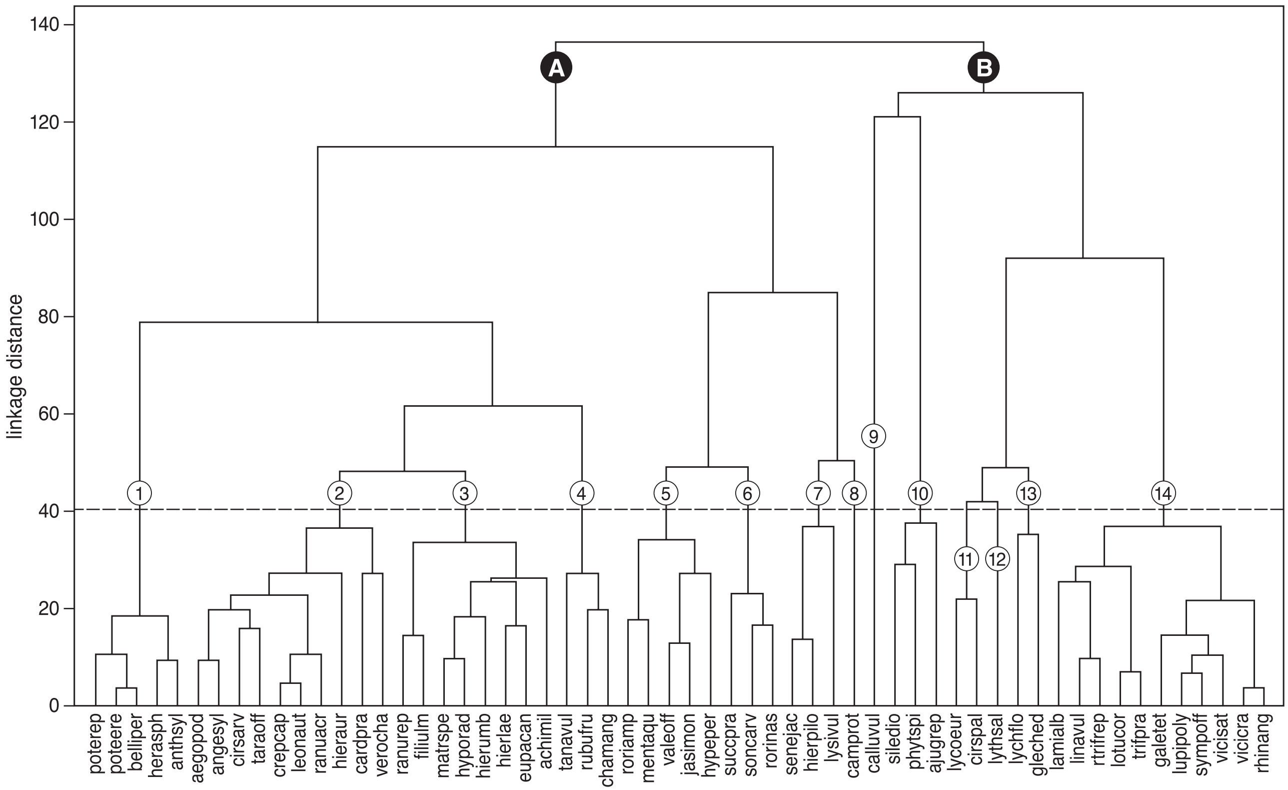


Fig. 2.1. Dendrogram of 58 plant species of the cluster analysis with euclidian distances, based on insect visitor data. The dotted line is the cut of point for determining seperate clusters, based on the amalgamation schedule (see text). The clusters were given numbers, indicated on the lines. The two major branches were given the letters A and B.

The type head was mainly in the clusters 2 and 3, and brush was distributed over several clusters. The flower types tube, gullet and flag fall entirely into branch B (table 2.3, fig. 2.2). Only five out of twenty plant species with other flower types fall into these clusters (4x brush, 1x head). In clusters 13 and 14 all plant species have the flower types tube, gullet or flag. The plant species with actinomorphic flowers are in both cluster branches (table 2.3). In branch A and the clusters 9, 11 and 12 all species are actinomorphic. All zygomorphic flowers are in cluster branch B (clusters 10, 13 and 14). In cluster branch A most flowers have an upwards orientation (table 2.3), except *Campanula rotundifolia* (downwards) and *Chamerion angustifolium* (sideways). All other plant species with a sideways orientation of flowers are in cluster branch B. There is only one plant species with an entirely upwards orientation in cluster branch B (*Cirsium palustre*, cluster 11).

Diptera (flies, syrphids and *Rhingia*) and Apoidea (solitary bees, bumblebees and honeybee) combined constituted more than 75% of the visitor individuals for all plant species (fig. 2.3), except for *Achillea millefolium* (62%), *Lythrum salicaria* (67%) and *Veronica chamaedris* (72%). Most of the plant species in branch A have high proportions of flies, syrphids or solitary bees (fig. 2.3), whereas honeybees, bumblebees and *Rhingia* dominate in branch B. The clusters are characterised by the following visitor group compositions:

In cluster 1, more than 75% of the visitors are flies. In cluster 2, the proportion of flies is still >50%, but >25% of the visitors belong to other insect groups. In cluster 3, 50-75% or more of the visitors are flies and syrphids combined, with flies >30% and syrphids <50%. In cluster 4, flies are a quarter to a third of the visitors, but almost 50% of the visitors are honeybees and bumblebees. In the clusters 5, 6 and 7, syrphids are the most abundant group: in cluster 5, they constitute around half of the visitors, the other half is a mixture of other groups. In cluster 6, syrphids are around three quarters of the visitors. A high proportion of solitary bees (mostly oligolectic species) characterises cluster 7 (>25% bees) and *Campanula rotundifolia*, the only species in cluster 8 (>50% bees). More than 75% of the visitors on *Calluna vulgaris*, the only species in cluster 9, are honeybees. In cluster 10 the dominant visitor is *Rhingia* campestris. Cluster 11 and 12 may also be considered as one cluster, as the split is just above the cut-off point (fig. 2.1). The plant species in these two clusters have a mixture of different visitor groups; *Lythrum salicaria* (cluster 12) had the largest proportion of butterflies of all plant species. In cluster 13, almost 85-90% of the visitors are *Rhingia*, bumblebees and the honeybee combined. Cluster 14 is dominated by bumblebees: 75%-100% of all visitors.

The most frequently observed insect groups are flies, syrphids (incl. *Rhingia campestris*) and bumblebees. Solitary bees and the honeybee constituted more than 50% of visitors for only one plant species each (*Campanula rotundifolia* and *Calluna vulgaris*, respectively). Beetles, ants and wasps did not represent a majority of visitors for any plant species, and are concentrated in clusters 1 and 2 (ants and wasps, mainly on Apiaceae) and beetles also in clusters 1, 2, 3 and 10. Plant species where butterflies constituted >10% of the visitors were distributed over many clusters: *Eupatorium cannabinum* (3), *Mentha aquatica* and *Jasione montana* (5), *Cirsium palustre* (11), *Lythrum salicaria* (12), and *Lotus corniculatus* and *Trifolium pratense* (14). Moths were most abundant in cluster 2 (*Cardamine pratensis* and *Veronica chamaedris*). These were mainly small moth

Table. 2.4. Plant linkage levels (mean L_p ±SE) per cluster, means were calculated from values in table 2.3.

cluster	N plant species	N flower types	total L_p	mean L_p
1	5	3	58.8 ±26.6	4.7 ±1.7
2	10	3	54.2 ±9.8	4.6 ±1.1
3	8	4	63.8 ±10.3	4.2 ±0.8
4	4	3	67.0 ±11.7	5.2 ±1.3
5	5	2	39.8 ±7.4	4.3 ±0.7
6	3	2	18.7 ±3.8	4.2 ±0.6
7	3	3	26.7 ±10.7	3.1 ±0.8
8	1	1	12	2.0
9	1	1	24	3.6
10	3	3	8.0 ±3.8	2.2 ±0.6
11	2	2	18.0 ±7.0	2.3±0.2
12	1	1	46	3.8
13	2	2	12.5 ±0.5	1.7 ± 0.1
14	11	3	9.4 ±1.6	1.7 ± 0.1

species, only on *Lythrum salicaria* (11) and *Lychnis flos-cuculi* (12) larger moth species were present with >5%.

The plant species with the highest total linkage levels (between 90-120 insect species) and mean linkage levels (between 8- 13 insect species) are the four Apiaceae species in clusters 1 and 2 (table 2.3). The total linkage levels of *Hieracium laevigatum* and *Hypochaeris radicata* (cluster 3) are also among the highest (90-100 species), but the mean linkage levels fall into the intermediate range (3-6 insect species). Two other plant species with high mean linkage levels are *Eupatorium cannabinum* in cluster 3 (around 9 species) and *Tanacetum vulgare* in cluster 4 (around 8 species). The mean linkage levels of the six species with the highest mean linkage levels are almost 2-10 times higher than those of the other species (table 1). Species in the clusters 1-7, 9 and 12 have a wide range of mean linkage levels (1.8-12.7 visitor species). The plant species in the clusters 8 and 10-14 all have low linkage levels (≤ 2.5 visitor species), except *Phyteuma spicatum* ssp. *nigrum* in cluster 10. The extremes in linkage level between the plant species average out when the means are calculated per census (table 2.4). Still, the clusters 1-6 have the highest, 7-12 intermediate and 13 and 14 the lowest plant linkage levels.

DISCUSSION

Characteristics of the clusters

The cluster-analysis produced distinct clusters or classes of plant species: the clusters differed in visitor frequencies, and the flower types were largely separated. Flies, syrphids and solitary bees on the one hand, and the proportion of bumblebees, *Rhingia* and honeybees

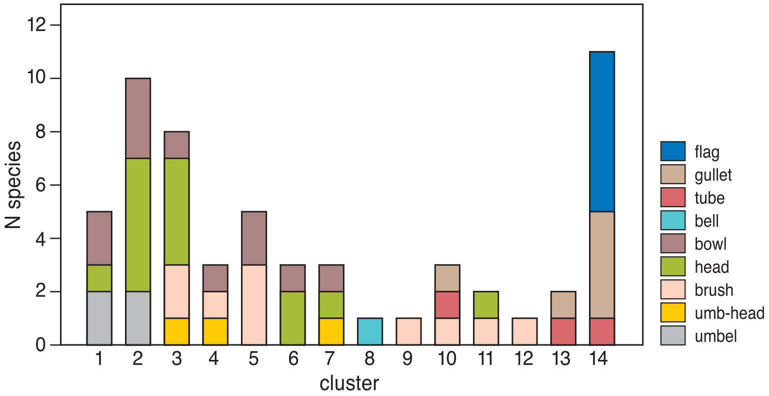


Fig. 2.2. The number of plant species with a certain flower type per cluster.

on the other were the main characteristic groups for the two branches A and B. In many clusters of plants, one or two insect groups contributed to the majority of visitor frequencies: flies (clusters 1 and 2), flies and syrphids (cluster 3), syrphids (clusters 5 and 6), syrphids and solitary bees (cluster 7), solitary bees (cluster 8), honeybees (cluster 9), *Rhingia* (cluster 10), bumblebees and honeybees (cluster 13) and bumblebees (cluster 14). The remaining clusters 4, 11 and 12 had a mixture of visiting groups. Diptera and Apidae s.l. are the majority of flower visitors in temperate Europe (Ellis & Ellis-Adam 1993; Elberling & Olesen 1999; Memmott 1999; Totland *et al*. 2000), this is confirmed by our own observations (fig. 2.2): in all plant species in this study they constituted more than 65% of the visitors. Lepidoptera (butterflies and moths) had a maximum of one third of the visiting individuals, but for only two plant species: *Lythrum salicaria* and *Veronica chamaedris*. The other insect groups were all below one fifth of the visitors for all plant species.

Plant species with the flower types umbel, umbel-head, bowl and bell were all in branch A, and also most of the species with brush and head. All species in branch A have actinomorphic flowers and nearly all have an upward orientation. Branch B contains all species with the types tube, gullet and flag and all zygomorphic species. Most species in branch B have a sideways orientation.

Accessibility of rewards

The accessibility of rewards, nectar and pollen, was the most important feature that determined the difference of the visitor groups between branch A and B, and also between the clusters. The main reason for an animal to visit a flower is the primary attractant, i.e. something the visitor profits from in an energetic or reproductive way. Therefore the type and accessibility of the primary attractants play a major role for which insects will visit a flower (Fægri & van der Pijl 1979). For the plant species in the present study, pollen and/or nectar, and in one case pollen and oil (*Lysimachia vulgaris*), were the primary attractants. Visitor assemblages, and therefore the classification of pollination types, are primarily determined by the accessibility of nectar (Corbet 2005; Stang *et al*. 2005). Our study confirmed this: the flower types tube, gullet and flag, which require from the visitor long and thin mouth-parts, and/or force to reach the nectar, are all in the same major branch B (fig. 1.1 and 1.2). The main visitors of these plant species are bumblebees and *Rhingia campestris*, species with relatively long probosces compared to the other insect groups. In an earlier analysis we had grouped *Rhingia* with the other syrphids, but that produced a less clear clustering than the presented one. Therefore *Rhingia* was considered as a separate group in our analysis.

Within the insect groups there are considerable differences in proboscis length. Among bumblebees, *Bombus hortorum* has the longest proboscis and *B. pascuorum* and *B. terrestris* queens are intermediate (Heinrich 1979; Fussell & Corbet 1992). They were the most frequent visitors on *Rhinanthus angustifolius, Symphytum officinale, Linaria vulgaris*, and *Galeopsis tetrahit. Bombus terrestris* (workers) is one of the species with short proboscis, similar in length as the honeybee, and cannot reach the nectar in these flowers in a legitimate way. *Bombus terrestris* is capable of biting holes into corolla tubes and visit flowers as nectar robbers. In this way it also facilitates access to nectar for honeybees, which cannot bite holes themselves. Most of the solitary bees in northern and central Europe have short probosces (Fægri & van der Pijl 1979), explaining why they predominantly visited plant species in branch A and only on plants with short corollas in branch B (e.g. *Lycopus europaeus*).

Variation in proboscis length exists also among syrphids other than *Rhingia* (de Buck 1990; Ellis & Ellis-Adam 1994a): the larger syrphid flies (mainly *Eristalinae* like *Eristalis intricarius* and *E. tenax*) can reach nectar in longer corollas than the smaller species (e.g. *Syrphus* species). The majority of syrphids visiting the plant species with head and brush flowers were *Eristalinae*; in clusters 5 and 7 more than 50% and in 6 more than 75%. Flowers of *Mentha aquatica, Valeriana officinalis, Jasione montana* and *Succisa pratensis* have relatively narrow corolla tubes of intermediate length (2-4 mm), which may explain the relatively higher proportion of bumblebees, *Rhingia* and butterflies. This may also be the case for *Eupatorium cannabinum*, but the larger proportion of flies (>40%) may indicate that nectar is more easily accessible in this plant species than in the plants mentioned before. Overall, the proportion of Diptera with shorter probosces (most flies and smaller syrphids) is largest in the clusters 1, 2 and 3. In cluster 1 are only plant species with open nectaries (bowl and umbel), and all umbel species are in clusters 1 and 2.

It is striking that butterflies hardly visited the deep flowers of plant species in cluster B (fig. 2.2). A reason may be that they have a preference for plants with clustered flowers and large nectar quantities, especially butterflies with larger body weights (Corbet 2000a). Most of the plants in cluster 14 that offer this, lack good landing facilities, a prerequisite for butterflies (Fægri & van der Pijl 1979). *Eupatorium cannabinum* (cluster 3) was the most popular plant species for butterflies. It flowered at the peak emergence of mainly *Inachis io* and invasion of *Vanessa atalanta* and *V. cardui* (Nymphalidae). So despite the fact that butterflies generally have rather long probosces, they were most frequently seen on plants with rather shallow corollas. This was also observed in coastal dunes in the Netherlands and in southern Spain (M. Stang, pers. comm.).

The effect of nectar accessibility on visitation could also be observed in closely related plant species. For example, the two *Cirsium* species have the same flower type (i.e., head), but are in different clusters: *C. palustre* (cluster 11) is mainly visited by insects with intermediate to long probosces, viz. bumblebees, butterflies, *Rhingia* and honeybee (fig. 2.2). It has longer tubes than *C. arvense* (cluster 2), which is mainly visited by flies.

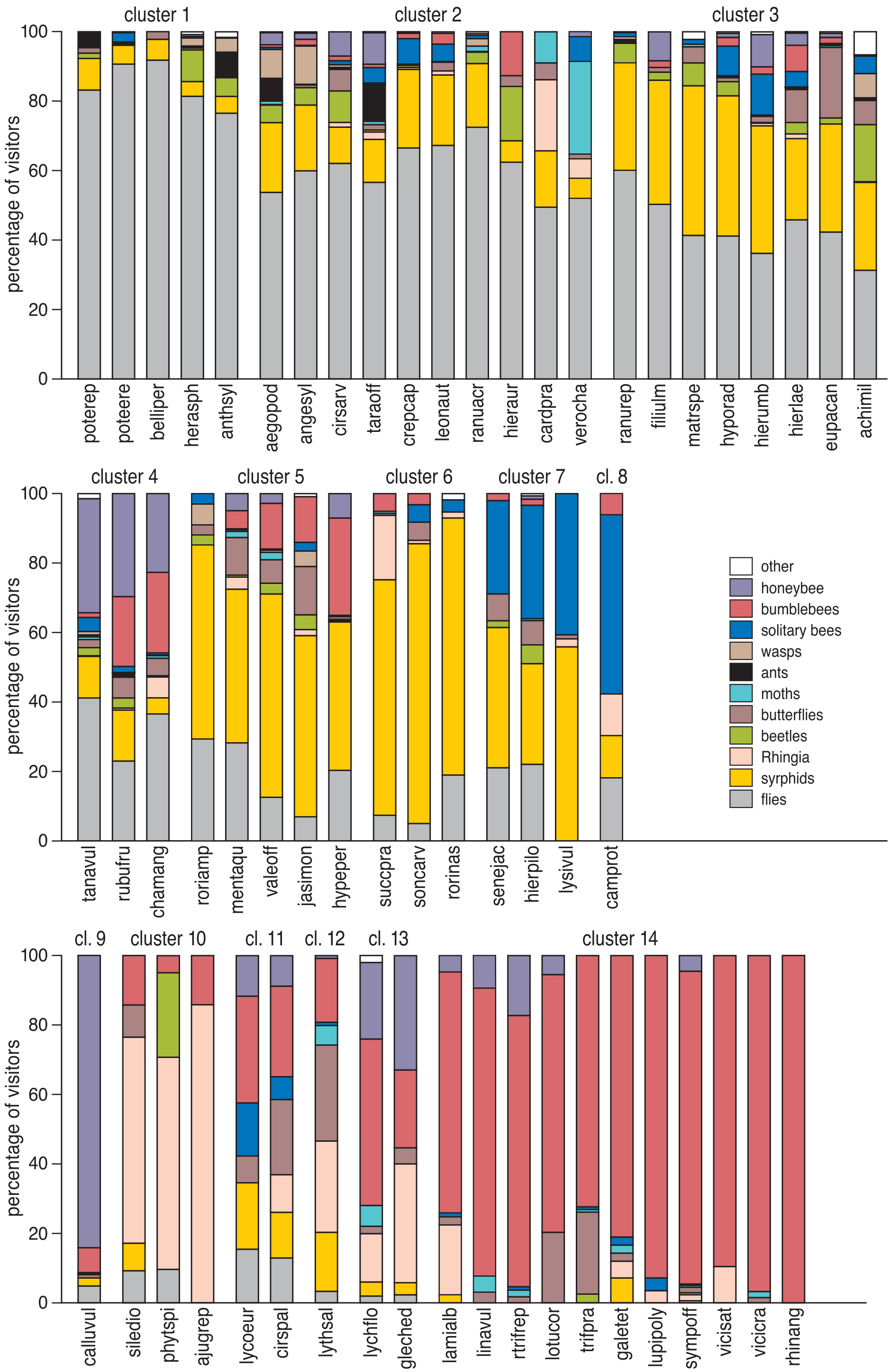
In addition to flower morphology, the chemical composition of nectar, sugar concentration and nectar production rate are factors that may determine the visitor composition. Insect species have various metabolisms and therefore also energy and nutrient needs (Fægri & van der Pijl 1979; Corbet 2000a, 2005; Dupont *et al*. 2004). This, apart from nectar depth, may be one explanation for why the flower types brush, head, umbel head and partially bowl are distributed over many clusters. Another reason may be the presence of other attractants.

Pollen is another primary attractant and is an important food and protein source for beetles, syrphids and bees (Fægri & van der Pijl 1979; Waser *et al*. 1996). Beetles and syrphid flies directly eat it, whereas bees (all three groups) mainly collect it for their offspring, often in large quantities (Fægri & van der Pijl 1979; de Buck 1990; Westrich 1990; Ellis & Ellis-Adam 1993; Goulson 1999). In several species the sparse amount of pollen is hidden (e.g. in *Rhinanthus angustifolius*). Consequently, even bee species with long probosces may visit different plant species for pollen and for nectar. The bumblebees and honeybees that were visiting yellow Asteraceae species (*Hieracium* spec., *Hypochaeris radicata* and *Sonchus arvensis*) mostly did this to collect pollen. Some of the plant species in clusters 13 and 14 were visited by few small syrphids, though only in small numbers. They were feeding on the pollen of the plant, sometimes even from the stigmas, and therefore do not play a role as structural pollinators.

Specialisation and generalisation

The degree of specialisation varied strongly between the 58 plant species in this study, as reflected by the plant linkage levels (L_p): the plant species with the highest linkage levels were visited by 20 (total L_p) or 8 (mean L_p) times more insect species than the plant species with the lowest L_p. Some plant species had relatively high total linkage levels, but intermediate mean linkage levels: this is largely due to the fact that they flowered during a long period, or flowered again after mowing. The latter plant species were visited by many insect species for all sites and periods in total, but locally and at a certain moment only by a few.

Considering linkage levels, the composition of functional visitor groups and the accessibility of primary attractants, the plant species in the clusters 10, 13 and 14 (nearly all tube, gullet and flag flower types) and *Lysimachia vulgaris* (see below) may be regarded as ecologically the most specialised. These 17 species constitute almost 30% of the analysed plant species in our community. Although this is a considerable amount, still the majority of plant species can be regarded as generalised. This is in concordance with other studies stressing the generalised nature of plant-pollinator interactions (Ellis & Ellis-Adam 1993; Waser *et al*. 1996; Memmott 1999). We know of only one community-based study in which the relation between the number of visitor species was related to flower features


cluster 1
cluster 2
cluster 3
percentage of visitors
100
80
60
40
20
0
poterep
poteere
belliper
herasph
anthsyl
aegopod
angesyl
cirsarv
taraoff
crepcap
leonaut
ranuacr
hieraur
cardpra
verocha
ranurep
filiulm
matrspe
hyporad
hierumb
hierlae
eupacan
achimil
cluster 4
cluster 5
cluster 6
cluster 7
cl. 8
percentage of visitors
100
80
60
40
20
0
tanavul
rubufru
chamang
roriamp
mentaqu
valeoff
jasimon
hypeper
succpra
soncarv
rorinas
senejac
hierpilo
lysivul
camprot
other
honeybee
bumblebees
solitary bees
wasps
ants
moths
butterflies
beetles
Rhingia
syrphids
flies
cl. 9
cluster 10
cl. 11
cl. 12
cl. 13
cluster 14
percentage of visitors
100
80
60
40
20
0
calluvul
siledio
phytspi
ajugrep
lycoeur
cirspal
lythsal
lychflo
gleched
lamialb
linavul
rtrifrep
lotucor
trifpra
galetet
lupipoly
sympoff
vicisat
vicicra
rhinang

and where these features of the flowers and insects were actually measured: Stang *et al*. (2005) showed that nectar holder sizes (depth and width of flowers) and the number of flowers per plant explained 71% of the variation in the number of visitor species in southern Spain. In that study, plants with shallow flowers often had more flowers per plant, and apart from the accessibility of nectar, the number of flowers was correlated with plant linkage level. Also in our study, plant species with composed inflorescences consisting of many small and shallow flowers had the highest linkage levels (up to 123 insect species in total or 12.7 species per census): the flower types umbel, umbel-head, brush and head.

The 17 specialised species may also be called "eutropous": they have concealed nectar and can only be visited by highly adapted (eutropous) visitors (table 2.2). Those visitors are species with long probosces, i.e. mainly bumblebees and *Rhingia campestris*, and to a lesser extent honeybees and butterflies. The plant species with flower types umbel and bowl in the clusters 1, 2 and 3 (table 2.3) can be considered to be allotropous: They have simple flowers with openly accessible nectar, and are mostly visited by flies, wasps and beetles. These insects often have other food sources than flower products (for example *Empis* species and the dung fly *Scathophaga stercoraria*) regularly catch other insects on flowers, and can therefore be regarded as allotropous, too. The umbelliferous Apiaceae were the most generalised as they were visited by a tremendous number of individuals and species. The remaining plant species may be regarded as hemitropous: they have intermediate accessibility of nectar, and syrphids and solitary bees as main visitors. However, the limits between hemi- and eutropous are less straight-forward: Many syrphids, all bees and most Lepidoptera primarily feed on flower products. But only the larvae of bees also depend on flower products, while the larvae of the other species eat something else. Bees are the only group with morphological pollen collecting facilities, and are the most adapted to flower visitation. Following this definition, all syrphids are hemitropous and all bees are eutropous. However, the proboscis length and learning abilities vary within all groups, and therefore also the capability to access "eutropous" flowers. From this one may conclude that most syrphids and solitary bees are hemitropous, and bumblebees and *Rhingia* campestris eutropous. For plant species the problem is similar: Is *Cirsium palustre* eutropous? Due to its longer tubes it certainly is more eutropous than *Cirsium arvense*, which again is less allotropous than the Apiaceae species.

The discussion above about specialisation mostly concerned nectar, but most of the specialised insect species (oligolectic bees) are specialised on pollen. Thus here we encounter a striking discrepancy: the specialisation of most plant species in our system is based on nectar, while the specialisation of insects is based on pollen! Pollen specialists have specialised on a food source which is mostly unconcealed (Fægri & van der Pijl 1979; Waser *et al*. 1996). In our study these food sources are e.g. Asteraceae species,

Fig. 2.3. Visitor compositions of the 58 plant species in this study. The insect species were divided into 12 groups (see text), shown as percentage of the total number flower visitors in 2000 and 2001. See table 2.3 for N values. The cluster numbers correspond to those in fig. 2.1 and table 2.3. Clusters 1-8 (upper two graphs) belong to the main cluster branch A, clusters 9-14 (lowest graph) to branch B.

Campanula rotundifolia and *Lysimachia vulgaris*. Many of the oligolectic bee species in this study visited generalist plant species, e.g. Asteraceae. This and the fact that the specialist plants are visited by generalist bumblebees is the reason for the asymmetry between plants and insects concerning the distribution of generalists and specialists (Jordano 1987; Bronstein 1995; Waser *et al*. 1996; Memmott 1999; Olesen & Jordano 2002). This has consequences for the vulnerability of plants to pollinator extinction: the vulnerability depends on the degree of specialisation of both the plant and the pollinators (Ashworth *et al*. 2004).

Only four plant species had a proportion of solitary bees that was high enough to be in a separate cluster (clusters 7 and 8). Two of these, *Lysimachia vulgaris* and *Campanula rotundifolia*, were visited by a single specialist bee species. The first is also the only example of a one-to-one species pollination system in our study: *L. vulgaris* and the oligolectic bee *Macropis europaea*. These flowers do not produce nectar, but oil (Vogel 1976; Vogel 1986). Instead of hiding the nectar physically, the plant uses another product than nectar to restrict the number of flower visitors. Apart from the bee, it was visited by several, mostly small, hoverfly species. The syrphid flies only feed on pollen and hardly touch the stigmas. The bee, however, collects pollen and oil, and touches the whole flower. It has a much higher visitation rate than the syrphids, and an individual bee visits more than 20 times as many flowers per unit time (M.M. Kwak, unpublished data). As the plant does not produce nectar, the bees will have to get it from other plant species. We have observed it on *Lycopus europaeus, Mentha aquatica* and *Lythrum salicaria*, all plant species with undeep and easlily accessible nectar. Apart from *M. europaea* there is one other oil collecting bee in Europe, *M. fulvipes* (Fægri & van der Pijl 1979; Rasmussen & Olesen 2000). This species is restricted to a limited number of places in the Netherlands, and never was very common (Peeters *et al*. 1999). The number of potential pollinators therefore is very restricted for *L. vulgaris*.

Campanula rotundifolia has a bell flower with a downward orientation and is difficult to land on for many insects. Insects must possess a learning behaviour to be able to deal with complicated flowers, like solitary bees, bumblebees and *Rhingia*, or must be small enough to land on the flowers and creep in, like very small flies (Muscidae and Tachinidae) and syrphids (Fægri & van der Pijl 1979). The main visitors were solitary bees, bringing more than 75% of the visits (fig. 2.3). The bees were exclusively oligolectic foragers on Campanulaceae: *Melitta haemorrhoidalis* and (only once) *Chelostoma rapunculi*. In northwest Europe, several oligolectic bees from different genera are specialised on species from the genus *Campanula* (Westrich 1990; Peeters *et al*. 1999). Is *Campanula* therefore also ecologically specialised? This will be only local specialisation, since in different areas *Campanula* had visitor compositions with and without solitary bees, but with successful pollination (Bingham & Orthner 1998; Blionis & Vokou 2001).

The categorisation of allo-, hemi- and euphilous species (table 2.2) is a way of describing ecological specialisation, based on the accessibility of the primary attractant (nectar) and adaptedness (proboscis length) of pollinators. It would be useful to add more features, something which has been suggested and initiated by Corbet (2005). But since the degree of (ecological) specialisation is continuous (Johnson & Steiner 2000; Ollerton & Watts

2000), it will be difficult to frame it into three distinct classes. Furthermore, this entomology-based classification will only be useful for insects and insect-pollinated plant species. In order to be generally valuable for a larger area than northern Eurasia, it should also include features from biotic pollination systems with other animals than insects.

In general, the type(s) of pollinators in combination with the number of visiting species and individuals may be a better way of expressing ecological specialisation than just the number of species. Reliance on a single functional type of pollinator is a far more widespread form of specialisation than reliance on a single pollinator species (Johnson & Steiner 2000).

Pollination syndromes in this study

Based on the observed visitor frequencies and the flower features, two of the pollination syndromes from table 2.1 are applicable for a part of the plants: the syndrome of flies (*myophily*) and the syndrome of bees (*mellitophily*). Most of the plant species in clusters 1 and 2 and some in cluster 3 have features that belong to the syndrome of flies: they are actinomorphic, simple, regular and shallow, have easily accessible nectar, light colours (whitish or yellow) and exposed sexual organs. And indeed, the main visitor group is flies, and also other unspecialised flower foragers like wasps. Most of these flowers are umbels or bowls, but also some shallow head-flowers may belong to this type: *Crepis capillaris* and *Hieracium aurantiacum*. The "head" flowers with slightly deeper tubes, *Cirsium arvense, Hieracium* spec. and *Leontodon autumnalis* were mainly visited by larger Muscidae and related families, and also Empididae that have longer probosces (but Empids mainly use them to eat other insects). Therefore they do not fit into the typical syndrome of flies.

The *melittophilous* (syndrome of bees) plant species can be found in cluster 14, and partially in clusters 10 (*Ajuga reptans*) and 13 (*Glechoma hederacea*). They possess zygomorphic flowers, that all have a sideways orientation, depth effect, and strongly concealed nectar in either deep tubes or spurs, and/ or some have to be forced open mechanically. Only *Symphytum officinale* is actinomorphic and has a downwards orientation and does therefore not entirely fit into the typical syndrome. The main visitors were larger bees (bumblebees and honeybees), *Rhingia campestris*, and some butterflies. *Phyteuma spicatum* subsp. *nigrum* may also belong to this syndrome, even though it is not zygomorphic and has also some short-tongued visitors: its flowers have a sideways orientation, it is dark blue, and nectar and pollen are hidden (Kwak 1993; Kwak & Vervoort 2000). Solitary bees hardly visited any of these melittophilous plant species. As stated by Fægri & van der Pijl (1979) (table 2.1), solitary bees visited a range of different other plant species, mainly flowers with easily accessible nectar. *Campanula rotundifolia* and *Lysimachia vulgaris* are exceptions to this accessibility, as discussed above. Similar to our study, in a community of temperate grasslands in northern Japan the only "true" syndrome that could be identified was the syndrome of bees, with a close association of gullet and flag flower types with bumblebees (Nakano & Washitani 2003). Solitary bees mainly visited generalist plant species. Like in our study, in the Japanese grasslands the flower types umbel and bowl were mainly visited by non-syrphid Diptera.

One plant species, *Scrophularia nodosa*, was visited by wasps (*Dolichovespula sylvestris*), but had too few visitors in total for the analysis. Despite the fact that it is a typical example of a wasp flower (Fægri & van der Pijl 1979), it was frequently, and maybe mainly, visited by bumblebees and honeybees (F. Hoffmann, pers. obs.).

Two plant species, *Silene dioica* and *Lychnis flos-cuculi*, had features of *psychophily*, i.e. the syndrome of butterflies. They have actinomorphic, pink flowers with deep tubes, a rim allowing butterflies to land on, and a largely upwards flower orientation. However, the most abundant visitors were intermediate- to long-tongued bumblebees (*Bombus pascuorum* and *B. hortorum*), *Rhingia campestris*, and honeybees (*Apis mellifera*). Although butterflies visited these flowers, they were a small minority. Community studies in other temperate systems could not identify butterfly-syndromes either (Dicks *et al*. 2002; Nakano & Washitani 2003). As we only studied plant species during the day, we have no measures of night-active moths. However, no plant species was found with features of *phalaenophily*, the syndrome of moths (table 2.1). Hovering day-active moths, such as the migrating *Autographa gamma*, could occur in large numbers locally. They visited similar flowers as bumblees, *Rhingia campestris* or butterflies.

Beetles were most abundant on plant species that were mainly visited by flies. Plants with flowers large enough to come close to *canterophily* (syndrome of beetles) are *Rubus* cf. *fruticosus* and the *Ranunculus* species, but they lack most of the other *canterophily* features (table 2.1).

At least 50% of the plant species studied are generalist and do not fit in any of the traditional pollination syndromes. These plants mostly had moderate linkage levels, actinomorphic flowers with an upwards orientation, and the flower types head and brush. The main visitors were syrphids, solitary bees and honeybees, but to a lesser extent also members of all the other insect groups. Several of these plants are autogamous or apomictic: the species in the genera *Potentilla, Rubus, Taraxacum, Crepis* and *Hieracium*. In spite of this, they may still depend on insect visitation or pollination. It is known that sometimes cross-pollination can or must occur, and that the process of apomixis may be initiated by insect visitation (Fægri & van der Pijl 1979; Hoffmann *et al*. 2003).

Our approach to classify plant species using visitor data is largely from the point of view of the insects. It shows the flower preferences of insects, but not their pollination quality, and the latter matters for the plant (Kwak *et al*. 1998; Johnson & Steiner 2000; Ollerton & Watts 2000). However, our method provided a large amount of data in a fairly short time: to study the qualities of all major insect species for all plant species in a community is a daunting task, though not impossible. For our analyses we chose to use the number of individuals per insect group rather than the number of species. The number of species gives a different pattern of insect composition for many of the clusters (fig. 2.4): in clusters 1 and 2, the role of wasps (particularly Parasitica and Symphita) would be much larger than of flies. Other large (positive and negative) differences can be seen for syrphids (clusters 4, 5 and 6), solitary bees (clusters 7 and 8) and for honeybees, *Rhingia* and bumblebees in general (mainly clusters 8 - 14). It is unknown how the pollination efficiency of insect groups would alter the patterns even further.

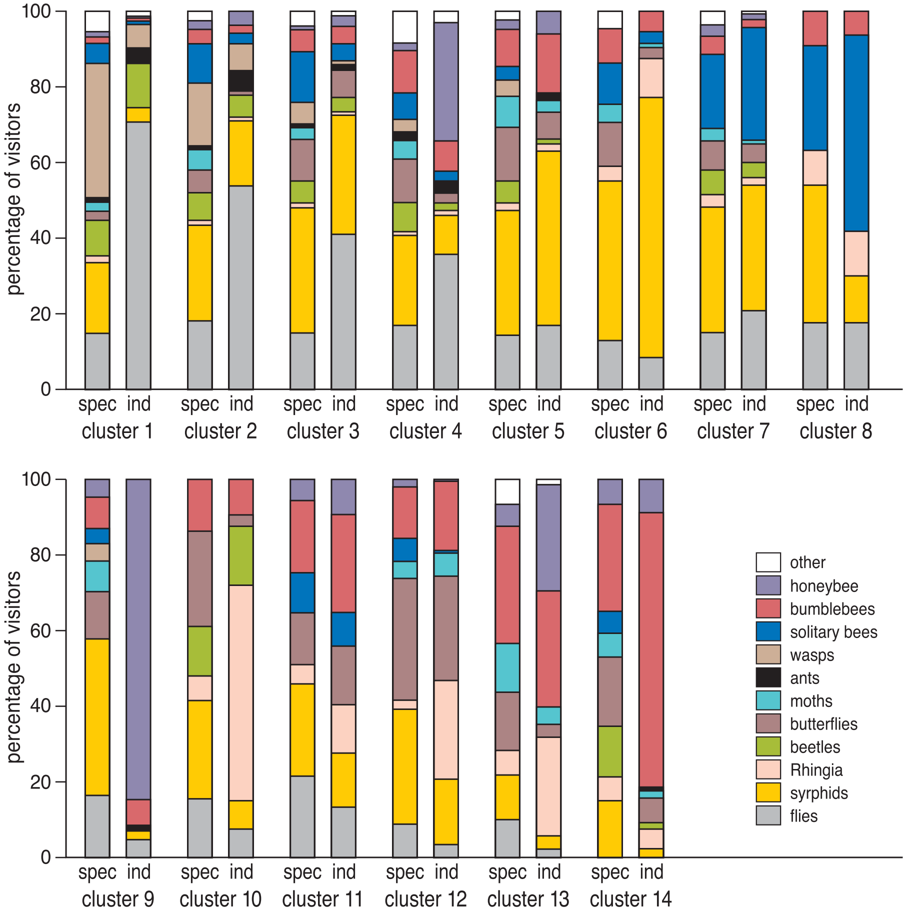


Fig. 2.4. The mean composition of insect groups for the 14 identified clusters based on the number of insect species (spec) and the number of insect individuals (ind) per plant species. The latter is the mean from fig. 2.2. (see fig. 2.1, table 2.3).

It is likely that many plant species have several best pollinators, rather than one single species. For example, the pollinator quality of syrphids for *Succisa pratensis* and the related *Scabiosa columbaria* appears to be similar to that of bumblebees, but the quality of *Rhingia*, flies and butterflies is much less (Kwak 1993). Butterflies are poor pollinators in general for European plant species (Jennersten 1984; Velterop 2000). Different visitors may play different functional roles for pollination, like long-distance pollen dispersal (gene flow) or high local pollen deposition. There can be differences in pollinator compositions within a plant species across larger geographical ranges, e.g. for *Scabiosa columbaria* (Kwak, unpub. data) or *Campanula rotundifolia* (Bingham & Orthner 1998). Furthermore, when the best pollinator is absent, the second best becomes the best (Armbuster *et al*.

2000; Ollerton & Watts 2000), thereby counterbalancing selection pressures for flower traits favouring only the best pollinator. *Rhingia* might play this role of the second best alternative in some plant species when bumblebees are not available.

Problems and solutions for pollination syndromes

The major criticism on the applicability of pollination syndromes is the restricted validity (Jordano 1987; Ellis & Ellis-Adam 1993; Waser *et al*. 1996; Memmott 1999). For at least 50% of the plant species in this study, syndromes may still have to be defined. For oil-producing flowers, that are much more common in tropical than in temperate climates, this may already be the case: they are well described and qualified as a pollination syndrome (Vogel 1976, 1986; Rasmussen & Olesen 2000). The flower types brush and head may be classified as "syrphid and solitary bee flowers", or as "the generalist syndrome". There is a lack of (published) data to evaluate existing and to define new syndromes (Johnson & Steiner 2000; Ollerton & Watts 2000). Both data of visitor frequencies and pollinator effectiveness for many plant species are needed (Fægri & van der Pijl 1979; Armbuster *et al*. 2000). To date, there is no study that included visitation and efficiency data for a plant community (Waser *et al*. 1996; Armbuster *et al*. 2000; Ollerton & Watts 2000).

The classic pollination syndromes and earlier works (Proctor 1978; Fægri & van der Pijl 1979) are a profound basis for a classification, but need extension and refining. This includes the terminology, as it is regarded as misleading and unsatisfying (Ollerton & Watts 2000). Because biotic pollination involves two parties, the plant and the pollinators, a classification system and the terminology should include both these parties. The syndrome of bees, for example, is also valid for animals other than bees, but within bees only for the larger or long-tongued species. Another problem is that different plant species allow different solutions concerning flower morphology to have the same pollinator assemblage: e.g. *Symphytum officinale* in our study and *Narcissus pseudonarcissus* (Proctor & Yeo 1973) have predominantly bumblebees as pollinators, but are not typical bee flowers as described in table 2.1. The term long-tongued bee-flowers with hidden nectar may be applicable, with subtypes such as zygomorphic flowers with sideways orientation, and actinomorphic, deep flowers with downwards orientation. As the type and accessibility of the primary attractants are one of the primary determining factors for the mode of pollination, it is logical to include this in the name. Plant species with biotic pollination are in a multidimensional continuum of pollination systems; pollination syndromes should therefore be defined broadly enough (Armbuster *et al*. 2000; Ollerton & Watts 2000). To structure the continuum of pollination syndromes, it will be useful to quantify flower features and flower visitor features empirically and group the taxa afterwards, using cluster analysis like in the present and other studies (e.g. Ollerton & Watts, 2000) or compartment analysis (Dicks *et al*. 2002). An example of empirical measurement are the matching of nectar-holder depth and width and the number of flowers per plant with insect proboscis lengths, body sizes, and the number of visitor species (Stang *et al*. 2005). The empirical approach may be a more neutral one than grouping flower types *a priori* and defining syndromes based on taxonomy.

ACKNOWLEDGEMENTS

We would like to thank Maaike de Vlas and Tineke Schwab for assistance in the field, Theo Peeters, Wouter van Steenis, Kim Meijer, Kobus Boeke and Riek van Noordwijk, and late Henny Wiering for helping with insect identification, and Martina Stang for advise on the interpretation of the results and the discussion. This study was funded by the Stimulation Program Biodiversity of the Netherlands Organization for Scientific Research-Earth and Life Sciences (NWO-ALW).

3

Diversity of flowering plants and their insect visitors in habitat fragments surrounded by various types of land use

Frank Hoffmann, Manja M. Kwak and Jelte van Andel

SUMMARY

In agricultural landscapes, values for nature conservation are concentrated in highly dynamic, mostly linear landscape fragments, like road verges and ditch banks. Many plants depend on insect pollination, therefore knowledge of the diversity of plants and their pollinators in such habitats is important for conservation. We analysed the effect of the type and intensity of land use ("landscape diversity": low, intermediate and high) on insect visitation of wild plant species, and the relation between these communities. The diversity and abundance of flowering plants and flower-visiting insects were investigated in road verges and ditch banks in the Netherlands in 2000 and 2001. The number of plant species at a site was significantly affected by the surrounding landscape diversity. Total insect diversity was not affected, but solitary bees and wasps had significant differences between sites differing in landscape diversity. The number of insect species was positively correlated with the number of plant species and flowers at a site. Site management (mowing) had no significant effect on plant species richness for a whole season. The species richness and abundance of solitary bees was higher at sites that were not mown or mown with flowers in adjacent areas than at mown sites without flowers in adjacent areas. Flowering plants and flower-visiting insects are strongly affected in agricultural landscapes. Fragmentation and land use affect plant species richness and abundance, and together with site management these affect insect species richness and abundance. Particularly solitary bees, the most specialised group, are vulnerable. Increasing habitat heterogeneity in agricultural areas will have a positive effect on insect species richness. These insects are needed for pollination of wild plants, and several crops.

INTRODUCTION

The visitation of flowers by pollinating insects is crucial for the reproduction and maintenance for almost 70% of angiosperm plant species (Kearns & Inouye 1997). Changes in land use like agricultural intensification, habitat fragmentation, and invasions of alien species have led to the decline of species. The number of plant species and populations as well as the density within populations have declined, e.g. in the Netherlands (Plate *et al*. 1992). Also several flower visiting insect taxa have declined in many countries, e.g. butterflies (Dover *et al*. 1990; Thomson 2001), bees (Westrich 1996; Benedek 1997; Peeters *et al*. 1999; Calabuig 2000; Cane & Tepedino 2001), and bumblebees (Williams 1986; Rasmont 1988; Kwak *et al*. 1996). Great concern exists for the consequences of pollinator fauna decline for wild plants and insect-pollinated crops (Corbet 1997; Tepedino *et al*. 1997; Kearns *et al*. 1998; Neal 1998; Corbet 2000b; Cox & Elmqvist 2000; Kremen & Ricketts 2000; Paton 2000; Roubik 2000). The lack of appropriate pollinators leads to a lower seed set and inbreeding (van Treuren *et al*. 1993, 1994; Kwak *et al*. 1996). Plants in fragmented habitats with small population sizes are especially vulnerable (Oostermeijer *et al*. 1994; Washitani 1996; Kwak *et al*. 1998; Luijten *et al*. 2000; Oostermeijer *et al*. 2000).

In agricultural landscapes, values for nature conservation are concentrated in highly dynamic, mostly linear landscape fragments, like road verges, ditch banks and field margins. For many flowering plant species these patches are a last refugium in heavily human-influenced landscapes. Furthermore, they are important for maintaining plant diversity outside nature reserves, and may function as corridors between the remaining natural areas (Kleijn *et al*. 1997; Kwak *et al*. 1998; Schaffers 2000; Velterop *et al*. 2000). In the Netherlands roadside habitats cover up to 2.1% of the total land area. Compared to the 4.2% of natural areas this is a considerable amount (Schaffers 2000). Flora and fauna in linear habitat fragments are affected directly through mowing regime, and indirectly from the surrounding matrix through spraying, and fertiliser input (Kleijn *et al*. 1997; Schaffers *et al*. 1998; Schaffers 2000).

There is an increasing interest in community processes in pollination ecology (Moldenke 1975; Memmott 1999; Dicks *et al*. 2002). To understand the importance of diversity for the functioning of plant- pollinator systems, research should take place at the community level: facultative interactions of low specificity are the rule and interactions have a high spatio-temporal variation (Jordano 1987; Ellis & Ellis-Adam 1993). Ideally, all flower-visiting species within a community should be studied, without any prior selection of species (Waser *et al*. 1996; Memmot 1999).

In this study we will investigate the effect of the type and intensity of land use on insect visitation of wild plant species in linear habitat fragments. We will present data on diversity and abundance of flowering herbs and flower-visiting insects in road verges and on ditch banks. We want to know (1) how diversity and abundance of plants and insects are influenced by "landscape diversity", i.e. land use and agricultural intensity, and (2) in which way these interacting communities are related with each other. We are specifically interested in which way taxonomically different flower visitor groups are affected and what consequences this may have for pollination and conservation. Even though it was not a goal of this study, we analysed the effect of management (mowing) on species richness of plants and insects as it was such a frequent aspect at most of the sites.

MATERIAL AND METHODS

Research area

We conducted our study in the province of Drenthe in the north of the Netherlands (ca. 53°00' N, 6°35' E), an area of pleistocene origin with (loamy) sand or peat on sand soils. We inspected ca. 325 km of road verges and ditch banks to select 51 linear, unshaded sites of 100 m length and 1-2 m width. Verges along large roads and motorways were avoided for safety reasons. After selection, the sites were subdivided into three types of landscape diversity (Dauber *et al*. 2003), based on the adjacent land use:

1) Low diversity (N=20): the surrounding land is characterised by intensive agriculture with annually ploughed, fertilised and sprayed (arable) fields or annually sown grasslands. Planted crops were cereals, potatoes, sugar beet, maize or ornamental plants, and a field with a (North-American) blueberry orchard. For flower-visiting insects only the blueberry orchard and a field of ornamental flowers were possible food sources.
2) Intermediate diversity (N=19): neighbouring permanent grasslands that are either grazed (cattle, sheep, horses) or mown, or the site is adjacent to a combination of types low and high. Possible food sources for insects are grasslands, meadows and hedges with mainly flowering shrubs like *Crataegus* and *Prunus* species.
3) High diversity (N=12): adjacent to the sites are found nature reserves and other natural areas, mostly hay meadows and wet heath within the National Park of the stream valley of the "Drentse Aa". Management activities are annual mowing or extensive grazing (cattle, horses). The hay meadows are rich in flowering species and together with hedges they are possible food sources for flower visiting insects.

Transect observations

Within each site the number of flowering units was counted of all occurring entomophilous plants (no graminoids or other wind-pollinated plant species). Flowering units were single flowers, umbels, heads and spikes, depending on the species. All insects visiting inflorescences, without any prior selection of taxa, were counted by walking slowly along a transect once. Insects were identified in the field to species or morphotype, henceforward "species" indicates both. We collected insects only occasionally, as this could disturb the observations. Samples of insects were identified later. The observation time of insects was usually between 11:00 and 16:00 h local time. We started and/or stopped earlier or later when forced by circumstances like extreme temperatures, light period, or abrupt weather change. The weather circumstances were as constant as possible, i.e. winds speeds less than 5 Beaufort, and no precipitation. In 2000, all 51 sites were visited approximately every two weeks from the beginning of May until the beginning of October, 10 rounds in total. Rounds were grouped into cohorts. In order to estimate annual effects, we investigated 18 of the sites again in 2001 from the beginning of May until the end of August (7 cohorts, N sites: low 7, intermediate 5, and high 7).

Calculations

Species richness (the number of species) and abundance (number of flower units or insect individuals) of plants and insects were summed per year for each site. For the 18 sites with observations in both years species richness was compared between the two years. For this purpose the Jackard's similarity index J was calculated: $J = a_{ij}/(a_{ij}+a_i+a_j)$, where a_{ij} is the proportion of species observed in both years, a_i in 2000 and a_j in 2001. Furthermore, we calculated the relative difference of species numbers between the two years for plants and insects, i.e. the percentage of species that are only found in one year relative to all species at the site.

We subdivided the plant species into nine flower types (chapter 2): umbel, umbel-head, brush, head, bowl, bell, tube, gullet, and flag. We calculated Shannon diversity indices (H'), an indicator that combines species number with abundance (Magurran 1988). $H' = \sum p_i \ln(p_i)$, where pi is the proportion of individuals of species "i". The insects were subdivided into nine groups, i.e. beetles, flies (non-syrphid diptera), syrphids (hover-flies), butterflies, wasps, solitary bees, bumblebees, honeybee, and remaining groups ("other"). The solitary bees were further subdivided into oligolectic (obligate specialists for one plant species or genus) and polylectic (unspecialized foragers) species.

Because flower species richness and the number of flower types were strongly correlated, we analysed the effect of two plant families with contrasting flower morphology (actinomorphic vs. zygomorphic), i.e. Apiaceae and Lamiaceae. We calculated the percentage of insect species that were only seen on these plant families.

Statistics

We used analysis of variance (ANOVA) to test effects of landscape diversity on plant and total insect species richness and abundance, flower type richness (the number of flower types) and H', and species richness of insect groups. Correlation was estimated between flowering plant species richness, flower abundance and the number of flower types (Pearson correlation coefficients). Plant species richness, flower type richness and flower abundance were added as covariables in the ANOVAs of the insect variables. Post hoc effects were tested with Tukey tests. Stepwise multiple regression was done with insect species richness as dependent and plant species richness, flower type richness and flower abundance as independents for those variables with significant effects in the ANOVAs. The per insect group abundances and effects of management on species richness of insect groups were tested with Kruskal-Wallis tests, because the variances were not distributed equally (Sokal & Rohlf 2001). We did not test honeybee species richness (only one species), and we did not test "other" at all (too few individuals per taxon). We tested the difference between the total number of insect species and the number of species minus the insect species that were observed on the Apiaceae or Lamiaceae with two-tailed t-tests. All tests were performed with the data summed per site for the whole year. However, we also tested per cohort effects of landscape diversity on species richness and abundance of plants and insects with ANOVAs. As the number of sites was very low compared to 2000 the above described statistical tests were not done for the data from 2001. They were only used for comparison of phenology and estimate the effect of annual effects. All statistical tests were performed with the package SPSS for Windows version 12.0.1 (2003).

RESULTS

Species numbers and annual variation

The total number of flowering plant species found was 97 from 24 families in 2000, and 74 species from 20 families in 2001. We found 361 insect species from 9 orders in 2000, and 204 species from 8 orders in 2001. The similarity between years was higher for plants than for insects, expressed as *Jaccard*-index and as relative difference of species: plant mean *Jaccard*-index= 0.6, insect mean *Jaccard*-index= 0.4. The relative difference between years (the percentage of species that are only found in one year relative to all species at a site) was lower for plants (7.3%) than for insects (15.1%). Plant and insect species richness and abundance both have a peak in high summer (Mid-July until the end of August) in 2000 (fig 3.1 a-d). Insect species richness and abundance had another, lower peak at the beginning of the season (Mid-May). In 2001 (fig. 3.1 e-h) the May peak is about a week later.

Flowering plant diversity

Species richness of flowering plants and the number of flower types were higher in the landscape diversity type "high" than in "low" (table 3.1). Flower abundance did not differ between landscape diversity types. Flower species richness and flower abundance per site were correlated ($r= 0.49$, $p< 0.001$), as well as flower species richness and the number of flower types ($r= 0.70$, $p< 0.001$) and flower abundance and the number of flower types ($r= 0.33$, $p< 0.05$). Species richness of plants in 2000 is lowest in landscape diversity type "low" from 20 July - 14 August (fig. 3.1a), i.e. in cohorts six ($F_{2,40}= 19.4$, $p< 0.001$), seven ($F_{2,46}=3.8$, $p< 0.05$), eight ($F_{2,46}= 4.1$, $p< 0.05$) and nine ($F_{2,26}= 4.2$, $p< 0.05$). The diversity indices *H* of plants and insects (table 3.1) did not differ significantly between landscape diversity types, and were not correlated either.

Insect diversity

Total insect species richness and abundance did not differ between landscape diversity types (table 3.1), but it differed in cohort six (around 20 July, $F_{2,38}= 6.1$, $p< 0.01$; fig. 3.1c). Effects of landscape diversity were found on two insect groups (table 3.1): species richness and abundance of solitary bees were higher in types "intermediate" and "high" than in "low". Oligolectic bees were even stronger affected (fig. 3.3): in "low" less than 10% were oligolectic bees, whereas in "intermediate" and "high" this was more than a third of the species and individuals (species, analysis of variance: $F_{2,48}= 5.8$, $p< 0.01$, individuals, Kruskal-Wallis test: $X^2= 14.9$, $p< 0.001$). Insect abundance differed for

Table. 3.1. The number of species, abundance and Shannon diversity indices *H*' (mean ±SE) of flowering plants and flower-visiting insects in the landscape diversity types low, intermediate and high in 2000.

		landscape diversity			
		low (n^{1}= 20)	intermediate (n= 19)	high (n= 12)	statistics^{2}
flowering plants	N species	16.3 ±1.27^{a}	18.1 ±1.4^{ab}	23.5 ±1.8^{b}	F2,48= 5.819, p= 0.005
	abundance	7598 ±1363	10153 ±2208	9043 ±2208	n.s.
	H'	1.33 ±0.09	1.49 ±0.10	1.55 ±0.12	n.s.
	N flower types	6.6 ±0.3^{a}	6.8 ±0.3^{ab}	7.7 ±0.3b	F2,48= 3.211, p= 0.049
all insects	N species	42.8 ±4.4	46.4 ±3.6	54.3 ±6.6	n.s.
	abundance	500 ±129	416 ±88	447 ±91	n.s.
	H'	2.50 ±0.15	2.87 ±0.08	2.90 ±0.14	n.s.
beetles	N species	2.7 ±0.4	3.5 ±0.4	3.5 ±0.7	n.s.
	abundance	17.8 ±4.3	24.3 ±13.6	46.3 ±16.3	n.s.
flies	N species	8.3 ±0.9	10.21 ±1.2	12.0 ±1.4	n.s.
	abundance	307.1 ±118.7	222.8 ±62.8	297.3 ±78.1	n.s.
syrphids	N species	12.7 ±1.5	13.9 ±1.3	15.3 ±2.0	n.s.
	abundance	79.3 ±19.7	83.2 ±15.3	83.9 ±19.8	n.s.
butterflies	N species	4.8 ±0.7	4.6 ±0.7	5.4 ±1.4	n.s.
	abundance	13.6 ±3.6	12.1 ±2.5	21.3 ±6.6	n.s.
wasps	N species	5.1 ±0.9	2.7 ±0.6	5.4 ±1.3	n.s.
	abundance	13.5 ±3.0^{a}	4.8 ±1.2^{b}	13.8 ±4.0^{a}	X^{2}=6.571, p= 0.037
solitary bees	N species	2.0 ±0.5^{a}	4.7 ±0.8^{b}	5.8 ±1.0^{b}	$F_{2,48}$=7.218, p= 0.002
	abundance	3.8 ±1.2^{a}	14.9 ±4.1^{b}	14.5 ±3.7^{b}	X^{2}= 13.005, p= 0.001
bumblebees	N species	3.85 ±0.4	3.3 ±0.3	3.1 ±0.5	n.s.
	abundance	29.3 ±5.5	24.6 ±5.9	21.0 ±5.0	n.s.
honeybee^{3}	abundance	22.7 ±12.8	17.0 ±6.4	12.8 ±6.3	n.s.

[1]Number of sites. [2]Significances were tested with one-way analyses of variance or Kruskal-Wallis tests for abundances of insect groups; a different letter following the SE indicates significant differences; n.s.= no significant differences were found. [3]The honeybee (*Apis mellifera*) is only one species.

wasps, "intermediate" was lower than "low" and "high". For two other insects groups differences were found in cohort six (around 20 July): syrphids ($F_{2,38}$= 4.9, p= 0.012) and flies ($F_{2,38}$= 8.1, p= 0.001), data not shown.

Insect species richness was significantly affected by flower species richness and flower abundance (table 3.2): almost 50% of the variation of insect species richness can be explained by the two covariables, where flower species richness (fig. 3.2a) explains 43.2% of the variation, and flower abundance (fig. 3.2b) adds 5.1%. Flower species richness was related to species richness of beetles, flies and wasps, and flower species richness and abundance for hoverflies (table 3.2).

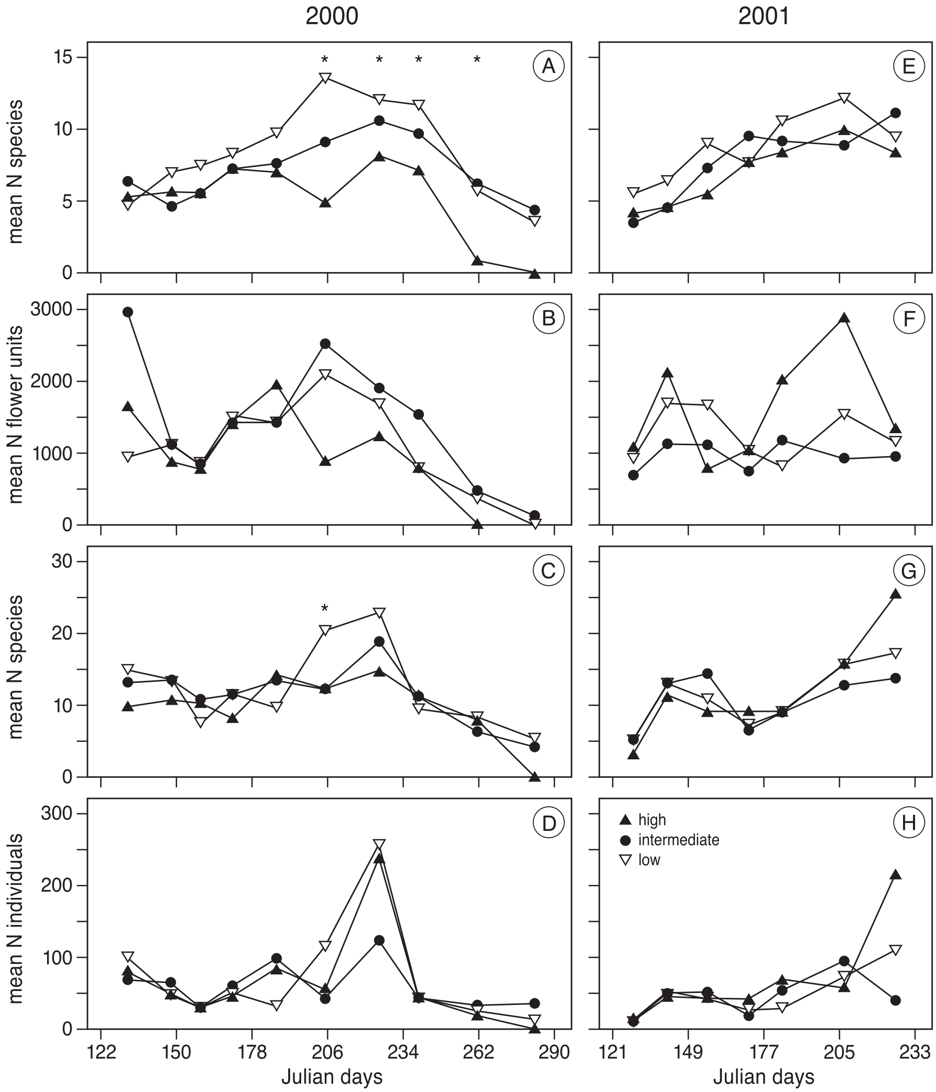


Fig. 3.1. Phenology of flowering plants and flower-visiting insects in the landscape diversity types low, intermediate and high, means per site. 2000: (A) number of flowering plant species, (B) number of flower units, (C) number of insect species and (D) number of insect individuals. 2001: (E) number of flowering plant species, (F) number of flower units, (G) number of insect species and (H) number of insect individuals. Julian days are continuous days from 1 January; day 122 (2000) and 121 (2001) are 1 May in both years (2000 was a leap year). Standard error bars are not indicated for the sake of clarity. Differences between landscape diversity types within cohorts were tested with univariate analyses of variance; * indicates significant differences at $\alpha = 0.05$.

Table. 3.2. Regression analyses of flower variables on insect species richness. Only the variables with significant effects in the analyses of variances (as covariables with landscape diversity as factors) were used for the regressions.

	flower species richness			flower abundance			regression model			
	β	t	p	β	t	p	r^2	df	F	p
all insects	0.52	4.5	0.000	0.28	2.4	0.019	0.48	2	24.4	0.000
beetles	0.45	3.5	0.001		n.s.		0.18	1	12.3	0.001
flies	0.51	4.2	0.000		n.s.		0.25	1	17.4	0.000
syrphids	0.38	3.2	0.002	0.42	3.5	0.001	0.46	2	22.4	0.000
wasps	0.38	2.9	0.006		n.s.		0.13	1	8.2	0.006
solitary bees	0.53	4.4	0.000		n.s.		0.27	1	19.2	0.000

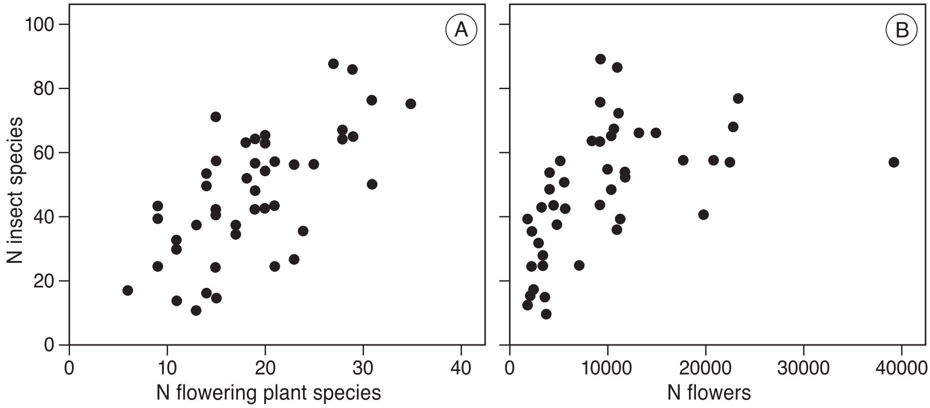


Fig. 3.2. Relation between flowering plant species richness and insect species richness (A) and flower abundance and insect species richness (B). A black dot indicates summed value for a whole year. The multiple regression and beta coefficients are indicated in table 2.

The percentage of insect species that were exclusively observed on Apiaceae was 18.6%, for Lamiaceae this was 0.6% (for all sites and both years). There was a significant difference between the total number of insect species observed on all plant families (46.7 ±2.7, mean ±SE per site) and the number of insect species per site minus those insect species exclusively observed on Apiaceae (35.7 ±2.1 t= -3.183, p< 0.005). This difference was not found for Lamiaceae: the number of insect species per site minus those insect species exclusively observed on Lamiaceae was 46.0 ±2.7, which is very close to the total number of insect species above (46.7).

Site management

The mowing regime of the various sites varied unexpectedly strongly and was unpredictable. Mowing frequency varied between zero and six times during the flowering season

Table. 3.3. Effects of mowing frequency (not mown, once, or twice and more during the flowering season) and the availability of unmown sites on insect abundance and species richness. "Mowing with escape" means that the area adjacent to a mown road verge or ditch bank is not mown (e.g. a meadow) or that there are unmown sites within a distance of 100 m.

		mean ±SE				
		no mowing	mowing with escape	mowing 1x	mowing ≥ 2x	statistics[1]
number of sites		18	14	7	12	
all insects	abundance	398.9 ±70.6	474.1 ±121.8	399.3 ±125.7	554.8 ±195.0	n.s.
	N species	46.1 ±4.7	48.9 ±4.4	38.9 ±8.2	49.3 ±6.4	n.s.
beetles	abundance	33.7 ±11.3	33.9 ±18.6	10.3 ±4.2	18.3 ±6.4	n.s.
	N species	3.0 ±.05	3.6 ±0.6	2.9 ±0.9	3.2 ±0.6	n.s.
flies	abundance	243.5 ±59.1	272.0 ±97.8	22.3.4 ±73.2	349.0 ±183.6	n.s.
	N species	9.94 ±1.2	9.5 ±1.1	8.6 ±1.3	10.9 ±1.7	n.s.
syrphids	abundance	85.4 ±17.2	73.9 ±14.1	45.6 ±14.8	106.3 ±31.1	n.s.
	N species	13.2 ±1.5	14.0 ±1.4	10.1 ±2.1	16.3 ±2.2	n.s.
butterflies	abundance	21.4 ±5.3	13.2 ±3.6	9.6 ±3.4	10.0 ±2.2	n.s.
	N species	5.8 ±1.0	4.6 ±1.0	4.0 ±1.1	4.3 ±0.8	n.s.
wasps	abundance	11.7 ±3.0	9.3 ±3.1	12.4 ±7.2	8.0 ±1.6	n.s.
	N species	4.2 ±0.9	4.1 ±1.0	4.1 ±1.9	4.5 ±0.9	n.s.
solitary bees	abundance	9.9 ±3.5ab	18.4 ±4.3b	4.4 ±3.8a	5.6 ±1.7a	X^2=11.99, p=0.007
	N species	3.7 ±0.6ab	5.7 ±1.0b	2.3 ±1.6a	3.1 ±0.9a	X^2=8.33, p=0.040
bumblebees	abundance	23.2 ±4.7	25.5 ±6.3	32.7 ±14.5	25.3 ±5.7	n.s.
	N species	3.2 ±0.4	3.7 ±0.5	3.6 ±0.8	3.6 ±0.5	n.s.
honeybee	abundance	14.3 ±5.1	12.3 ±5.4	48.7 ±35.8	16.3 ±7.2	n.s.

[1] Significances were tested with one-way analyses of variance for all insects and Kruskal-Wallis tests for insect groups; a different letter following the SE indicates significant differences; n.s.= no significant differences were found.

in 2000. Eventually also the not mown sites were mown after the research period in October or November. The type of mowing machinery and the removal of litter varied between the sites, mostly litter was not removed. Often a small strip of vegetation most distant from a road was not mown, and plants were still flowering at these sites. Sometimes only several hundreds of meters of a road verge were mown by a local land owner. For analysis, we subdivided our 51 sites into three categories: 18 sites were categorised as "not mown", including those mown at the end of the flowering season (end of August or September), 14 sites were categorised as "mowing with escape": despite mowing, possibilities for foraging were present as unmown strips, adjacent meadows with flowering plants or unmown sites within 100 m. Of the remaining sites, 7 were mown once, and 12 twice or more times. When a site was recently mown, the number of flowers was zero, therefore at these sites insects could not be observed, and these censuses were not used in the analyses.

There were no effects of mowing regime on total plant species richness and flower abundance, nor on total insect species richness and abundance. The number of solitary bee species and individuals was significantly higher in mown sites with unmown patches or meadows in the vicinity compared to sites where this was not the case (table 3.3): the number of individuals was four times higher and the number of species two times. Several plant species produced new flowers after mowing, usually within a month, but for the majority of species it was lower than the first flowering.

DISCUSSION

Spatial scale and diversity

In agricultural landscapes, diversity of flowering plants and flower-visiting insects are affected by a number of factors. For both plants and insects in these landscapes it is important that these factors are integrated in conservation measures. In our study, the two spatial scales landscape (land use and agricultural intensity) and site (plant diversity in a road verge or ditch bank), had different effects on the interacting communities: plant species richness was affected by landscape diversity, whereas insect species richness and abundance were mainly determined by flower species richness, and to a lesser extent flower abundance. Only in high summer was there a detectable effect of landscape diversity on insect species richness. Insects have different spatial (larger) and temporal (shorter) scales than plants (Sowig 1989; Sutherland *et al*. 1999): the effect of landscape and fragmentation on organisms is scale-dependent (Vessby *et al*. 2002; Chust *et al*. 2004). This may explain why insects in our study reacted differently than plants. An organism's perception of whether something is a boundary or not, or whether it is sharp or diffuse, is an essential parameter in understanding the effects of habitat fragmentation (Chust *et al*. 2004). Landscape diversity in our study can also be regarded in terms of habitat fragmentation, where "high" is the most continuous (natural) landscape type, and "low" the most fragmented. In this view, habitat fragmentation affects plants more strongly than insects: plants are sessile, and were more affected by landscape diversity than insects, which are mobile.

Support for the fact that *in situ* diversity is more important than landscape for insects comes from several studies: in California, grassland type and local habitat quality were the most important determinants for butterfly species richness, while landscape context had no effect (Collinge *et al*. 2003). Similar to our study, abundance varied widely, but in disturbed sites common species were dominant, whereas in higher quality sites the proportion of rare species was higher. In South-Africa insects were more sensitive to local habitat characteristics than fragment size (Donaldson *et al*. 2002). In Estonia, diversity and abundance of flowers and bumblebees was compared between semi-natural and agricultural habitat, the agricultural practices being less intensive than in western Europe. Species richness of bumblebees was lower in the agricultural sites, but abundance did not differ, indicating that more individuals of fewer species were present in agricultural sites. This is explained by a different flowering plant species composition rather than land use, with less plant species suitable for less bumblebee species (Mänd *et al*. 2002). The availability and abundance of certain dicotyledonous flowers was related to bumblebee species richness, density and abundance in Finland (Bäckman & Tiainen 2002). Similarly, Sutherland *et al*. (2001) found that the distribution and abundance of aphidophagous hoverflies was positively affected by flower abundance in the UK.

The bulk of flowers or insect individuals was often constituted by few species. This also explains the weaker effect of flower abundance on insects compared to plant species richness. Furthermore, some of the very abundant plant species were hardly visited at all, e.g. *Euphrasia stricta* or locally *Trifolium repens*. The early peak in flower abundance is largely due to *Anthriscus sylvestris* (Apiaceae). This coincided with the peak of insect species richness and abundance: almost one fifth of all insect species was observed on the Apiaceae including *A. sylvestris*. Especially flies and some beetles could be very abundant on the umbels of *A. sylvesris*. Also, around 75% of all wasp species were observed exclusively on this plant family. This also shows that functional diversity, i.e. the number of flower types, is important for insect diversity. Functional and species diversity of plants were highly correlated in our study.

The species compositions of insects differed more between the two years than that of plants: annual fluctuations of insects are larger compared to plants (Bronstein 1995). This is not surprising, as most of the plants were perennials, and insects usually live for only one season. Furthermore, many of the insect species that were only observed in one of the years occurred in small numbers, and had a low chance of being reobserved. The temporal difference of effects of landscape diversity can be largely due to phenology: earlier and later in the season there are fewer species, therefore the number of observations and power of statistical testing was lower then. If one is interested in measuring effects of land use or fragmentation on insect diversity, but is time-limited, it may be advisable to study only the most species-rich season. In our study summer (end of July and beginning of August) was the only period in which we found effects on plant and insect species richness within cohorts.

Because no significant effects were found on the Shannon diversity index, we think that species richness and abundance are more useful indicators for our study.

Differences between insect taxa

Separate insect taxonomical groups respond differently due to among others differences in life history and larval requirements. Solitary bees were the only group that was significantly affected during the whole season. For the other groups hardly any significant effects were found, only during the summer peak for syrphids and other Diptera.

Solitary bees and bumblebees depend on flower products during their whole life cycle, whereas most of the other insect groups do so only in the adult phase. Bumblebees are generalists, colonial bees that can forage at considerable distances from their nest, and even avoid its nearest vicinity (Saville *et al*. 1997; Osborne *et al*. 1999; Bäckman & Tiainen 2002; Dramstad *et al*. 2003). Solitary bees, however, mostly have a shorter phenology, must forage near to their nests, and are often food specialists (Kwak *et al*. 1996; Westrich 1996; Calabuig 2000). Around a third of the solitary bee species in the

Netherlands are oligolectic (Peeters *et al*. 1999); ideally this proportion should be reflected in our data. In the sites with landscape types "intermediate" and "high" this was the case. However, in "low" the proportion of oligolectic bees was less than 10% (fig. 3.3). This means that the specialist bee species are more affected than the generalists. Generally, bee faunas are very poor in agrarian landscapes where all semi-natural habitats have been removed, and where no core habitats such as pastures or forests exist in the vicinity (Calabuig 2000; Kleijn *et al*. 2001).

Adult syrphids depend on flowers (Kleijn *et al*. 2001), but contrary to bees they are said to have a high mobility (Sutherland *et al*. 2001). Therefore syrphids were much less affected than solitary bees as they could move much easier between sites differing in flower availability. It is surprising that we did not find an effect on butterfly species richness, since these are often thought to be good indicator species. The fact is that we almost exclusively found common (19) and migratory (4) species, only one was red-listed nationally. In the Netherlands, the majority of rare species only occurs in nature reserves (Dijkstra *et al*. 2003), and even common species have declined during the last decade of the 20th century (CBS *et al*. 2004). Overall, species richness of most groups tended to be lower in landscape diversity type low, though not significantly (table 3.1). The effect may be indirect through plant diversity, which was significantly lower.

Most insects depend on several habitat types to complete their life-cycle: the partial habitat concept (Westrich 1996). Sites for nesting may be located elsewhere than for foraging or mating, and larvae often need other food sources than adults. Habitat heterogeneity is important for insect diversity (Kearns & Inouye 1997; Wynhoff *et al*. 2001; Verberk *et al*. 2002). Chust *et al*. (2004) found differences in response to landscape for each dipteran functional group analysed. Different insect taxa may not be correlated with each other concerning occurrence of and effects of habitat, because the taxa are related to dif-

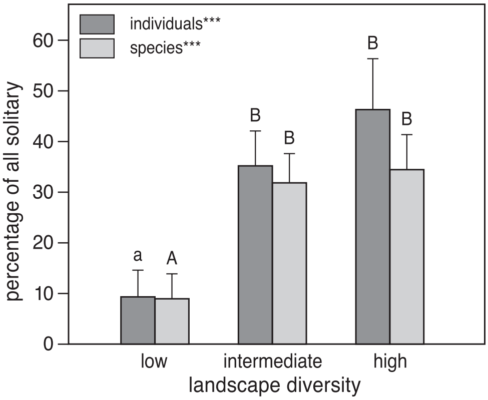


Fig. 3.3. Oligolectic bees in the landscape diversity types low, intermediate and high as percentage of individuals and species of all solitary bees in 2000. A different letter indicates a significant difference between types (small for individuals, capitals for species). Significance was tested with a univariate analysis of variance, *** means $p < 0.001$.

ferent kinds of heterogeneity (Vessby *et al*. 2002; Chust *et al*. 2003). In the landscape diversity type "low" the habitat was very homogeneous, with hardly any trees or shrubs. In "high" it was much more heterogeneous, another probable explanation for the difference in species richness between the landscape diversity types.

Site management

In the course of the season it appeared that much of the flowering vegetation was "pressed" into the edge of road verges or ditches. In several road verges and ditch banks individual plants, mostly *Urtica, Rumex* and Apiaceae, were sprayed with herbicides by farmers, or sometimes these plants were only chopped off. In both cases plants would not flower, thereby decreasing flower abundance. Repeated spraying of crop fields, some fields (potatoes) almost weekly, and of some grasslands, was an indirect effect on our sites. There were many parties involved that did not all know each other's policies and practices: local authorities, farmers and other landowners, state forestry, and water management authorities. Compared to national and provincial road verge management where species richness is stimulated, the local road verge management is very chaotic. We considered it as a current characteristic of the study area.

The management did not significantly affect flowering species richness or flower abundance over a whole year. Of course, directly after mowing no flowers were present at a certain site, but sites without flowers and therefore insects were not used for analysis. Furthermore, at several sites plants had started to make new flowers after mowing, or flowers were still present at the edges of a site. It is very likely that management effects on plants are not easily detectable within two seasons. The type of management (Schaffers *et al*. 1998; Schaffers 2002), and agricultural practices like fertilising and spraying (Andreasen *et al*. 1996; Le Cœur *et al*. 2002) are factors determining plant species richness over several years. It is known that delayed or no hay removal in road verges leads to nutrient accumulation and on the long run impoverishment of plant species richness (Schaffers *et al*. 1998). Many sites in our study were mown more than once, and mostly the hay was not removed.

Again, solitary bees were the only affected group: species richness was highest when there were alternative foraging sources close to a mown site. Mowing removed the bees' food sources for at least a week. Although many plant species were able to reproduce flowers after mowing (see box 9.1), this may be too late for bees and other insects. "Phenological mismatch" between flowering and bee flight period is fatal for bees, and may be for plants (Bronstein 1995). The other insect groups may be more mobile, or, similar as with plants, effects are more long-term. Several suggestions are made for conservation of pollinators: e.g. perennial plants for bumblebees (Fussell & Corbet 1992; Osborne & Corbet 1994), high floristic diversity (Corbet 1997, 2000b) and habitat heterogeneity as discussed above. The partial habitat concept clarifies that habitats should be preserved as a whole (Westrich 1996; Kwak *et al*. 1998; Calabuig 2000; Wynhoff *et al*. 2001). For bumblebees nest growth in an agricultural landscape is determined by the management of a much larger area than just where the nest is located (Goulson *et al*. 2001).

Pollination

The next question is what are the consequences of a low insect diversity for plant pollination. To answer this question, it is necessary to measure visitation rates, pollen deposition and seed set, as well as individual insect behaviour as related to flower community diversity and composition. To date, little is known about the effects (Corbet 1997; Kwak *et al*. 1998). A positive effect of a higher insect diversity is a higher chance of having the right pollinator. As we have seen, more plant species lead to more insect species. Plant species that depend on specialist species will suffer most from pollinator species loss: the oligolectic bees were the most affected group in our study. Plants may function as a "magnet" for other plant species and attract more insects (Laverty 1991). However, a higher plant diversity may also increase heterospecific pollen deposition on stigmas with its possible negative consequences for seed set (Kwak & Jennersten 1991; Kwak *et al*. 1998).

Agriculture may also benefit from insect diversity: e.g. for crop protection, as many insects whose larvae can control pest insects need flowers to forage on as adults.We found several parasite wasps on Apiaceae, although some possible pest species as well (e.g. Symphita wasps). More flowers also stimulate aphidophagous hoverflies (Sutherland *et al*. 2001). Apart from pest control, pollination of crops can also benefit from a high insect diversity. Apart from honeybees, we observed many bumblebees visiting the high-bush blueberry flowers in the orchard. They were probably important pollinators. From North America it is known that native bees are better pollinators of blueberries than introduced honeybees (Cane & Payne 1988). In the field with ornamental poppies (grown for their seed capsules) we observed many different insect species visiting the flowers. This ornamental crop may also benefit from insects in the area.

Conclusions

Our study has shown that flowering plants and flower-visiting insects are affected at different levels in agricultural landscapes. Fragmentation, land use and site management affect plant species richness and abundance, and together these affect insect species richness and abundance. Particularly solitary bees, the most specialised group, are vulnerable. Consequences of low insect species richness for pollination of plants are still to be revealed, but they are likely to be negative.

Conservation of flowering plants and flower-visiting insects in agricultural landscapes requires an integration of the direct effects from mowing and removing hedges, and indirect effects from land use, such as spraying and fertiliser input. Agricultural policies are about to change in the European Union, particularly in countries that joined in 2004. Therefore it is necessary to know how past agricultural changes have affected diversity and ecosystem processes, in order to provide guidelines for conservation and restoration policy and management. An example of a policy that does not achieve the conservation goals are agri-environmental schemes in Europe (Kleijn *et al*. 2001). It is crucial to use effective measures since they can cost a lot of money, but they should be evaluated ecologically. Monitoring has shown that measures are mostly too short-term to show a positive effect (Kleijn *et al*. 2001; Geertsema 2002; Manhoudt & de Snoo 2003; Kleijn & Sutherland 2004).

Management of linear habitat fragments can enhance plant diversity by using appropriate mowing regimes with hay removal within two weeks, to reduce nutrient accumulation. Plants must also have a chance to flower and set seed, thus the phenology of target species can be an important criterion for mowing regime. A higher plant diversity and flower availability are important for the conservation of flower-visiting insects. Increasing habitat heterogeneity in agricultural areas will have a positive effect on insect species richness, since many insects need several partial habitats for their life cycle. These insects are needed for pollination of wild plants, and several crops.

ACKNOWLEDGEMENTS

We would like to thank Maaike de Vlas and Tineke Schwab for assistence in the field, and Theo Peeters, Wouter van Steenis, Kim Meijer, Kobus Boeke and Riek van Noordwijk, and late Henny Wiering for helping to identify the insect species. This study was funded by the Stimulation Program Biodiversity of the Netherlands Organization for Scientific Research-Earth and Life Sciences (NWO-ALW).

4

Biodiversity affects plant-flower-visitor foodwebs

Frank Hoffmann and Manja M. Kwak

SUMMARY

Foodweb analysis can give insight into the consequences of biodiversity loss for ecosystems. In this paper a foodweb approach is used for plant-pollinator interactions. The questions are how connectance and linkage levels of plants and insects are affected by species richness, and whether effects differ between functional groups. Connectance is the proportion of all possible interactions between plants and insects that are actually established. Linkage level is the number of other species in a foodweb a particular species interacts with. In road verges and ditch banks differing in species richness in the Netherlands, the visitation of insect species on flowering plant species was investigated in 2000. Path analysis was used to investigate the effects of plant and insect species richness and abundances on connectance and linkage levels. The mean connectance per census was 0.21 ±0.01 SE, the mode 0.33. Plant species richness was the most important predictor in the path model. The mean plant linkage level per census was 2.27 ±0.09 SE and the mean total linkage level was 35.0 ±3.9 SE. The mean insect linkage level per transect was 1.29 ±0.02 and the mean total linkage level was 15.9 ±1.3. Plant linkage level decreased as there were more plant species, but there was a positive effect of insect species richness on plant linkage level that in turn was positively affected by plant species richness. Insect linkage level varied little, but the effect of plant species richness was positive, contradicting findings of other studies. The effects varied in strength and direction between taxonomical and functional groups of plants and insects. The study showed that human induced differences in species richness of plants and insects do affect network parameters of communities of flowering plants and flower visiting insects.

INTRODUCTION

During the last century, habitat fragmentation, invasions of alien species and changes in land use like agricultural intensification have lead and still lead to the decline of species. This is the case for flowering plants (Quinn *et al*. 1994; Tamis *et al*. 2004), and flower-visiting insects, e.g. butterflies (Dover *et al*. 1990), bees (Westrich 1996; Peeters *et al*. 1999; Calabuig 2000; Cane 2001; Peeters & Reemer 2003), and bumblebees (Williams 1986; Rasmont 1988). A pattern frequently observed for both animals and plants is that common species become more common and rare species rarer (Plate *et al*. 1992; Kwak 1994a).

Flowering plants and flower visitors are not isolated, but interact within communities. Foodweb analysis can be used to describe how communities are structured and can give insight in what may be the consequences of habitat fragmentation, climate change or bio-diversity loss for entire communities and ecosystems (Memmott 1999; Dunne *et al*. 2002; Owen *et al*. 2002; Garlaschelli *et al*. 2003). The study of bimodal mutualistic interaction webs, like between plants and flower visitors, and plants and frugivores, has recently gained increasing attention (Memmott 1999; Dicks *et al*. 2002; Olesen *et al*. 2002; Olesen & Jordano 2002; Bascompte *et al*. 2003; Dupont *et al*. 2003; Jordano *et al*. 2003). These mutualistic interaction webs differ from "normal" foodwebs in the fact that they consist of only two trophic levels with a more or less mutual relationship, i.e. plants (food source) and their flower visitors or fruit eaters (dispersal service). Insights are that plant-flower-visitor communities have a high level of generalisation, and that interactions between plants and flower visitors are weak and asymmetrical (Jordano 1987; Waser *et al*. 1996; Memmott 1999; Elberling & Olesen 1999; Olesen & Jordano 2002, but see Vázquez & Aizen 2003). This means that many specialised plant species are visited by generalised insects, whereas many specialised insects visit generalised plants. Furthermore, both levels of the web are dominated by a small number of abundant plant and animal (insect) species. In different communities, regardless of climate, the majority of insect species visits only two to five plant species on average (Olesen & Jordano 2002). Similarly, in networks many plant species have few interactions or no visitors at all, but only few species are visited by a large number of animal species (Moldenke 1975).

Two measures have been applied to quantify the interactions between trophic levels within a foodweb, viz. connectance and linkage level. Connectance is the proportion of interactions that are actually established relative to all possible interactions between the two levels (Jordano 1987). Linkage level is the number of species in a food web a particular species interacts with, and is often used as a measure of specialisation of a species or community (Olesen *et al*. 2002; Olesen & Jordano 2002; Dupont *et al*. 2003), and is also called niche width (Moldenke 1975; Heithaus 1979). The linkage level should be used in a food-web context, and the level of specialisation for the inherent number of taxa a species as a whole can interact with. For example, bumblebees potentially visit many more plant species than oligolectic (i.e. obligate specialist) bees, and can therefore be called more generalised. However, at a certain place and time they may have a low linkage level because they only visit a restricted number of plant species. Still, linkage level of a species can be used as an estimate for specialisation level within a large enough area or time span.

In a review about different bimodal network systems from different climates, Jordano (1987) found that as the number of species in a mutualistic system increases, the absolute number of established interactions also increases, but the connectance decreases exponentially. Similarly, in temperate climates connectance was marginally higher than the tropics, and much higher at higher altitude compared to lowlands (Olesen & Jordano 2002). This could be related to species richness, as in the tropics there are more species than in temperate regions, and at low altitudes there are more species than at higher altitudes.

Super-generalists are species that interact with many more species than the other species in the community (Olesen *et al*. 2002). On oceanic islands community linkage level and the occurrence of super-generalists of both plants and animals appeared to be much higher than on mainland habitats. Interestingly, Heithaus (1974, 1979) and Moldenke (1975) similarly found that in extreme habitats (deserts, alpine areas), the proportion of generalist flower visitors was higher and the chance of finding super generalists was expected to increase. More specialists were found under moderate climatic conditions. They also found that when plant species richness was higher, insect linkage level was lower. Contrary to this, Olesen & Jordano (2002) found that only plant species have higher linkage levels at higher latitudes and in lowlands, and lower levels at lower latitude, highlands and on islands. Insects did not show this pattern. This may be due to the fact that insects were analysed as a whole, while separate insect taxonomical groups may behave differently and should be compared within a group. In summary, species richness in extreme climates, at high altitude and on islands (isolation) is low. This can affect linkage levels and the balance between generalism and specialism.

In the studies described above, communities were compared over climatic and altitudinal ranges, and processes explained at evolutionary time scales. With this in mind we can ask ourselves whether lower linkage levels can also be found within systems where species richness has decreased because of human activities. Such sites may be comparable to the above mentioned oceanic islands and "extreme" climates: species richness has declined due to human activities, and many habitats are fragmented, comparable to islands.

The research questions in this paper are: (1) How are connectance and linkage levels of plants and insects affected by species richness, and (2) do effects differ between various functional groups of plants (flower types) and insects (taxonomical groups)? We predict that a lower species richness will have a negative effect on connectance and linkage levels. Flower visitation data from sites differing in species diversity caused by differences in land use will be used to answer this question, focusing on communities and functional groups, and not on individual species.

MATERIAL AND METHODS

Research area

We conducted our study in road verges and ditch banks. In agricultural landscapes these linear sites are often the only natural habitats. In the Netherlands road side habitats cover up to 2.1 % of the total land area, a considerable amount compared to the 4.2 % of natural areas (Schaffers 2000). The study area was situated in the Netherlands in the north of the province of Drenthe (53^{o}00'N, 6^{o}35' E), an area of Pleistocene origin with (loamy) sand or peat on sand soils. In 2000, we inspected ca. 325 km of road verges and ditch banks to select 51 linear sites of 100 m length and 1-2 m width. The sites were not shaded. Verges along large roads and motorways were avoided for safety reasons. The distance between sites varied between 500 m and 4 km.

The sites were situated in landscapes with different land use, varying from intensive agriculture (crop fields and sown grasslands), grazing meadows (cattle, sheep and horses) or semi-natural natural reserves. Plant species richness varied between sites that differ in land use (chapter 3). Insect species richness was affected by land use to a lower extend than plant species richness, but was positively related to plant species richness (chapter 3). In this paper we will concentrate on diversity and abundance of flowering plants and flower visiting insects.

Transect observations

Within each site the number of flowering units was counted of all occurring entomophilous plants (no graminoids or other wind-pollinated plant species). Flowering units were single flowers, umbels, heads and spikes, depending on the species. All insects visiting inflorescences, without any prior selection of taxa, were counted by walking slowly along a transect once. Insects were identified in the field to species or morphotype, henceforward "species" indicates both. We collected insects only occasionally, as this could disturb the observations. Samples of insects were identified later. The observation time of insects was usually between 11:00 and 16:00 h local time. We started and/ or stopped earlier or later when forced by circumstances like extreme temperatures, light period, or abrupt weather change. The weather circumstances were as constant as possible, i.e. winds speed less than 5 Beaufort, and no precipitation. All 51 sites were visited approximately every two weeks from the beginning of May until the beginning of October 2000, ten cohorts in total.

Variables

Our data set consisted of the number of flowers per plant species, and the number of individuals per insect species per plant species visited, all per site within cohort. The data set was used to calculate the following variables:

(1) Connectance (*C*), the proportion of all interactions realised relative to all possible interactions within a network; $C= N_i/S_p{\cdot}S_a$ where N_i= number of interactions between insect and plant species, S_p= number of plant species, S_a= number of animal (insect) species.

(2) Linkage level (L), the number of species of the other trophic level a species interacts with, where L_p= plant linkage level and L_a= animal (insect) linkage level. When a plant

species has L_p= 7 this means that this species was visited by seven insect species; L_a= 4 means that an insect species has visited four plant species. In this study the minimum insect linkage level L_a is always 1.0, as only insects were observed that visited flowers. The minimum plant linkage level L_p can be 0.0, as we counted all flowering plant species, including those that were not visited by insects.

For a number of analyses, separate functional or taxonomical groups of plant and insect species were used. We subdivided the plant species into nine flower types: umbel, brush, head, umbel-head, bowl, bell, tube, gullet, and flag (see chapter 2 for definitions). The insects were subdivided into nine groups: beetles, flies (non-syrphid Diptera), syrphids (hoverflies), butterflies, moths, wasps, ants, solitary bees, bumblebees, the honeybee, and other. Bees were subdivided into three groups: solitary bees, bumblebees, and the honeybee.

For each census (site within cohort) we calculated the connectance, mean plant linkage level L_p and mean insect linkage level L_a (i.e. mean over the linkage levels per species), and mean L_p and per flower types and mean La per insect group. The overall *mean linkage levels* with standard errors of the mean were calculated from these transect values. The *total linkage levels* were calculated as a mean over the total number of species a plant species was visited by, or an insect has visited during the whole research period and over all sites. For the total linkage levels we used only plant species that were visited by at least 25 insect individuals, and insect species with at least 25 individuals. We chose this (arbitrary) minimum to avoid outliers caused by the effect of incidental observations on the means.

Since mean connectance and L_p changed during the season, regressions of species richness on connectance and L_p were done within cohort first. As the results did not differ between cohorts, we used the values of the censuses of all cohorts together. We did not sum the interactions over the cohorts because this would create mistakes due to "phenological mismatching" (Jordano *et al*. 2003): this means that one would expect interactions between plant and insect species that are impossible, as their flowering and flight periods do not overlap.

Statistics

Analysis of variance (ANOVA) was used to test differences of linkage levels between categories of insect groups and flower types. We used multiple regressions to analyse relations between plant species richness and flower abundance as predictors and insect species richness and abundance as dependents.

Path analysis was used to test the effects of plant and insect species richness and abundances on connectance and linkage levels. This technique is useful for systems with many intercorrelated variables. Overall correlation of the variables can be decomposed into direct and indirect effects. Path coefficients (basically the standardised betas from multiple regressions) indicate the amount of change a dependent variable experiences from a change in the independent variables. Models are displayed in so-called path diagrams (Sokal & Rohlf 2001). Paths are indicated with arrows in such models. Exogenous variables are variables without external causes, endogenous variables are affected by exogenous and other endogenous variables. The fit of a path model can be tested using a chi-square goodness-of-fit test. Non-significance indicates that no deviation occurs between the observed correlations and the expected correlations calculated from the model, and that the model can be accepted as a valid explanation. One should be aware that several models may fit. Path analysis is a part of structural equation modelling (SEM) inside statistical packages (Mitchell 1993; Shipley 1997).

We used the SEM module SEPATH within the package Statistica version 6.1 (StatSoft, Inc., 2003) for the analyses of the path models. Models were tested for overall connectance, plant linkage level L_p and insect linkage level L_a and for L_p per flower type and L_a per insect group. For all variables several models were tested. Primarily, in all models plant species richness and flower abundance were used as correlated exogenous variables, and insect species richness and abundance as endogenous. As this did not provide any fit, the models were adjusted. If more than one significant model (i.e. $p > 0.05$) or when no fitting model could be found, the *Jöreskog GFI* and the *Akaike Information Criterion* in SEPATH were used to select the best model.

We tested the equality of slopes of plant species richness on L_a among insect groups in a general linear model (GLM), with L_a as dependent, insect group as independent and plant species richness as covariable. The interaction between insect group and plant species richness was significant, meaning that the slopes were not equal. Differences between the slopes from linear regression were then tested using a Tukey-test for comparison among slopes (Zar 1984).

The package SPSS 12.0.1. for windows (SPSS Inc., 2003) was used for all the other analyses than the path models.

RESULTS

Species diversity

In total, 79 flowering plant species and 361 flower visiting insect species were observed. There was a positive significant relation between plant species richness and flower abundance and insect species richness ($r^2 = 0.29$, $F=65.95$, $p < 0,001$) and abundance ($r^2 = 0.09$, $F=14.75$, $p < 0.001$, multiple regressions).

Species richness of flowering plants increased from ca. 6 species in spring to ca. 10 species in summer, and then decreased again to 6 species at the end of the season (fig. 4.1A). Flower visiting insects had a stronger increase and decrease: around 11 species in spring, then a slight decrease in June to 9 species, a summer peak of around 18 species, and a decrease to around 4 species in the last cohort.

Connectance

Connectance (the proportion of realised interactions of all possible interactions) showed an opposite phenological pattern compared to species richness and abundance: the mean connectance decreased from around 0.3 in spring to around 0.19 in summer, and then increased again to 0.3 at the end of the season (fig. 4.1B).The mean connectance per

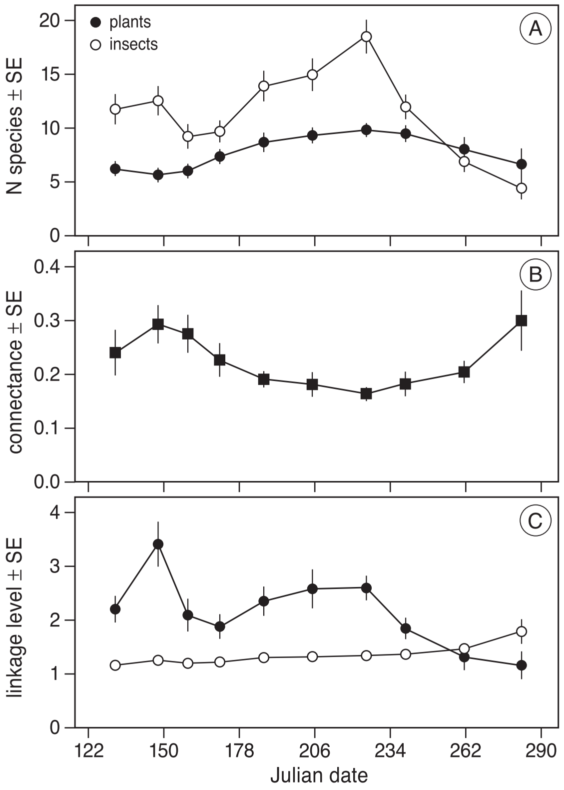


Fig. 4.1. Phenological patterns of species richness, connectance and linkage levels in 2000. (A) mean number of species of flowering plants and flower visiting insects, (B) mean connectance, and (C) mean plant and insect linkage level.

census was 0.21 ±0.01 SE and the mode 0.33. It varied between 0 (no interactions) and 1 (all plant species at a site during a census had interactions with all observed insect species). Connectances of 1.0 occurred only in 6 out of the 331 censuses, but in all 6 cases the number of plant species was also 1. In all other censuses connectance was lower than 0.58. Values of 0.0 (no insects observed on the flowers present) occurred only when plant species richness or flower abundance were very low (in 9 cases). The relation between connectance and plant species richness was negative and was best described with a power function ($r^2 = 0.90$, $F = 2767.86$, $p < 0.001$). Since linearity is an assumption of path-analysis, the variables were log-transformed. Connectance was not significantly related to insect species richness. In the path model for connection (fig. 4.2a) plant species richness was the most important predictor (table 4.1; $X^2 = 1.072$, df= 1, $p > 0.3$).

Plant linkage level

Plant linkage level L_p had a similar pattern as insect species richness: an increase towards summer (from 2.2 in May to 2.6 in July), and then decrease towards the end of the season (1.2 in October), but the highest peak was in May (3.4). The mean plant linkage level L_p per census was 2.27 ±0.09 SE and the mean total linkage level was 35.0 ± 3.9 SE. The only fitting path model had plant species richness and flower abundance as exogenous and insect species richness as endogenous variables affecting plant linkage level L_p (fig. 4.2b; X^2=2.288, df= 1, p> 0.1). The strongest effect was from insect species richness on L_p (positive). Plant species richness had a negative direct effect and a positive indirect effect through insect species richness. Therefore the net effect was rather small (table 4.1). A proposed model with a direct path from flower abundance L_p did not fit.

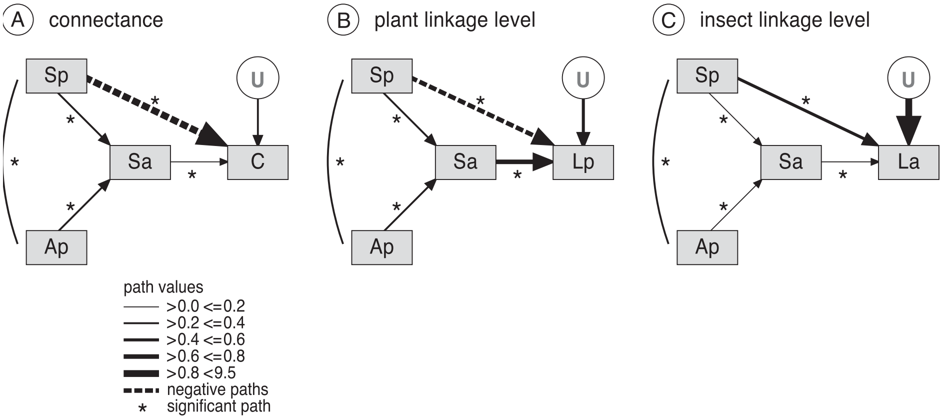


Fig. 4.2. Path models explaining variation Connectance (a), Plant linkage level (b) and Insect linkage level (c). Paths are indicated with arrows, correlations with unheaded lines. Thickness of lines corresponds with path values. For models (a) and (b) p>0.05. C=Connectance, Lp= plant linkage level, La= insect linkage level, Sp= plant species richness, Ap= flower abundance, Sa= insect species richness, Aa= insect abundance, U= residual (unexplained) variation.

Table. 4.1. Direct, indirect and total effects in the path analyses of connectance, plant linkage level and insect linkage level (see fig. 4.2 for the models). The total number of censuses was 331, the number of sensuses with insects was 322.

		Effects		
		direct	indirect	total
Connectance	Plant species richness	-0.981	0.035	-0.946
	Insect species richness	0.071		0.071
	Flower abundance		0.031	0.031
Plant linkage level	Plant species richness	-0.626	0.438	-0.188
	Insect species richness	0.889		0.889
	Flower abundance		0.382	0.382
Insect linkage level	Plant speceis richness	0.481	0.038	0.519
	Insect abundance	0.145		0.145
	Flower abundance		0.035	0.035

Flower type had a significant effect on mean L_p (table 4.2; F=49.7, df= 8, p< 0.001), the (partly overlapping) classes in order of decreasing average plant linkage level L_p were: 1. umbel (ca. 7 insect species per site), 2. umbel head, brush, and head (3 species), 3. bowl and bell (1 species), and 4. tube, gullet, and flag (<1 species).

Table. 4.2. Plant linkage levels (mean and total L_p) of flower types (see text), and direct, indirect and totel effects on mean L_p from path analyses (models in fig. 4.3).The total number of sensuses was 331.

flower type	mean L_p ±SE[1] (N censuses)	total L_p ±SE[2] (N plant species)	effects	direct	indirect	total
umbel	7.19 ±0.46	70.4 ±13.2	S_p[3]	-0.432	0.344	-0.088
	(250)	(5)	S_a[4]	0.726	-	0.726
			Nfl[5]	0.149	0	0.149
umbel head	3.10 ±0.37	39.9 ±4.1	S_p	-0.306	0.224	-0.082
	(121)	(3)	S_a	0.472	-	0.472
			Nfl	0.339	0.074	0.413
brush	3.10 ±0.30	31.6 ±7.2	S_p	-0.281	0.206	-0.075
	(154)	(7)	S_a	0.435	-	0.435
			Nfl	0.581	0.177	0.695
head	2.31 ±0.37	51.7 ±21.1	S_p	-0.284	0.173	-0.111
	(142)	(12)	S_a	0.366	-	0.366
			Nfl	0.380	0.103	0.483
bowl	1.39 ±0.12	44.2 ±7.8	S_p	-0.282	0.091	-0.190
	(277)	(9)	S_a	0.193	-	0.193
			Nfl	0.504	0.020	0.524
bell	0.92 ±0.28	12	S_p	0.324	0.196	0.520
	(25)	(1)	S_a	0.414	-	0.414
			Ap[6]	0	0.173	0.173
tube	0.85 ±0.11	13.5 ±1.5	S_p	-0.443	0.091	-0.351
	(118)	(2)	S_a	0.193	-	0.193
			Nfl	0.369	0	0.369
gullet	0.54 ±0.07	7.5 ±1.4	S_p	-0.249	0.051	-0.198
	(182)	(6)	S_a	0.108	-	0.108
			Nfl	0.262	0	0.262
flag	0.34 ±0.04	7.8 ±1.6	S_p	-0.174	0.070	-0.104
	(242)	(4)	S_a	0.148	-	0.148
			Nfl	0.388	0	0.388

[1] The mean of linkage levels over all sensuses within a flower type. [2] The mean over the total number of insect species a plant species was visited by during the whole research period (only plant species >24 visits are included). [3] Plant species richness. [4] Insect species richness. [5] Flower abundance per species. [6] Total flower abundance.

Two models had good fits for the L_p of several flower types: the first was the same as for overall L_p in fig. 4.2B, with total flower abundance as indirect exogenous variable. In the second model the abundance per flower type was used as exogenous variable affecting L_p directly and sometimes indirectly through insect species richness (fig. 4.3). For all flower types except bell the models with flower abundance per flower type provided the best fits. This means that a higher flower abundance increases the chance that a plant species is visited by more insect species.

The strengths of the paths between the variables differed between the flower types (fig. 4.3): they tend to be stronger for the types with a higher mean L_p (umbel, umbel head, brush, and head), and weaker for types with a lower mean L_p (tube gullet and flag). For brush, head, bowl, gullet and flag flower abundance had the strongest net effect on L_p (table 4.2). The effect of insect abundance on L_p was strongest for the four types with highest mean L_p (umbel, umbel head, brush, and head) and for bell. The total effect of plant species richness on L_p was lowest when the effect of insect species richness was strongest: the indirect effect of plants species richness works via insect species richness. The total effect of plant species richness compared to other variables within a model was strongest for bell (positive), and tube and gullet (negative).

Insect linkage level

The phenological pattern of insect linkage level L_a hardly changed at all (between 1.2 in May and 1.4 throughout the season) and was mostly 2.0- 2.5 times lower than plant linkage level, except at the end of the season (L_a= 1.8 in October; fig. 4.1C). The mean insect linkage level L_a per transect was 1.29 ±0.02 and the mean total linkage level was 15.9 ±1.3. For the overall L_a no fitting path model could be found. The model in fig. 4.3C is based on the models per insect group (fig. 4.4), with plant species richness and insect abundance as variables directly effecting L_a. The total effect of plant species richness on L_a was low compared to that on plant linkage level L_p (table 1).

The mean L_a (table 4.3) differed between insect groups (F=16.584, df=10, $p<0.001$,). The (overlapping) classes in order of decreasing mean L_a are: 1. fly, honeybee and bumblebee (around 1.4 plant species), 2. syrphid and beetle (1.3 species), and 3. butterfly, solitary bee, moth, ant, wasp and other (1.1 species).

For several insect groups fitting path models for L_a could be found (fig. 4.4). For almost all groups the path from per species abundance to L_a was significant, meaning that when more individuals of an insect species were observed, they were also observed to visit more plant species. It had the strongest total effect within a model for butterfly, moth, wasp, solitary bee, bumblebee and honey bee (table 4.3). The effect of plant species richness on L_a was the most important for beetle, fly and syrphid. There was a significant interaction effect of insect group and plant species richness (F=4.916, df=10, $p< 0.001$,). Significant differences of slopes of simple linear regression of the relation between plant species richness and L_a were found: fly and honey bee had the strongest slopes (b= 0.005), bumble bee, syrphid and beetle were intermediate (b= 0.004), and solitary bee weakest (b= 0.002). For the remaining groups the slopes were not significantly different from zero.

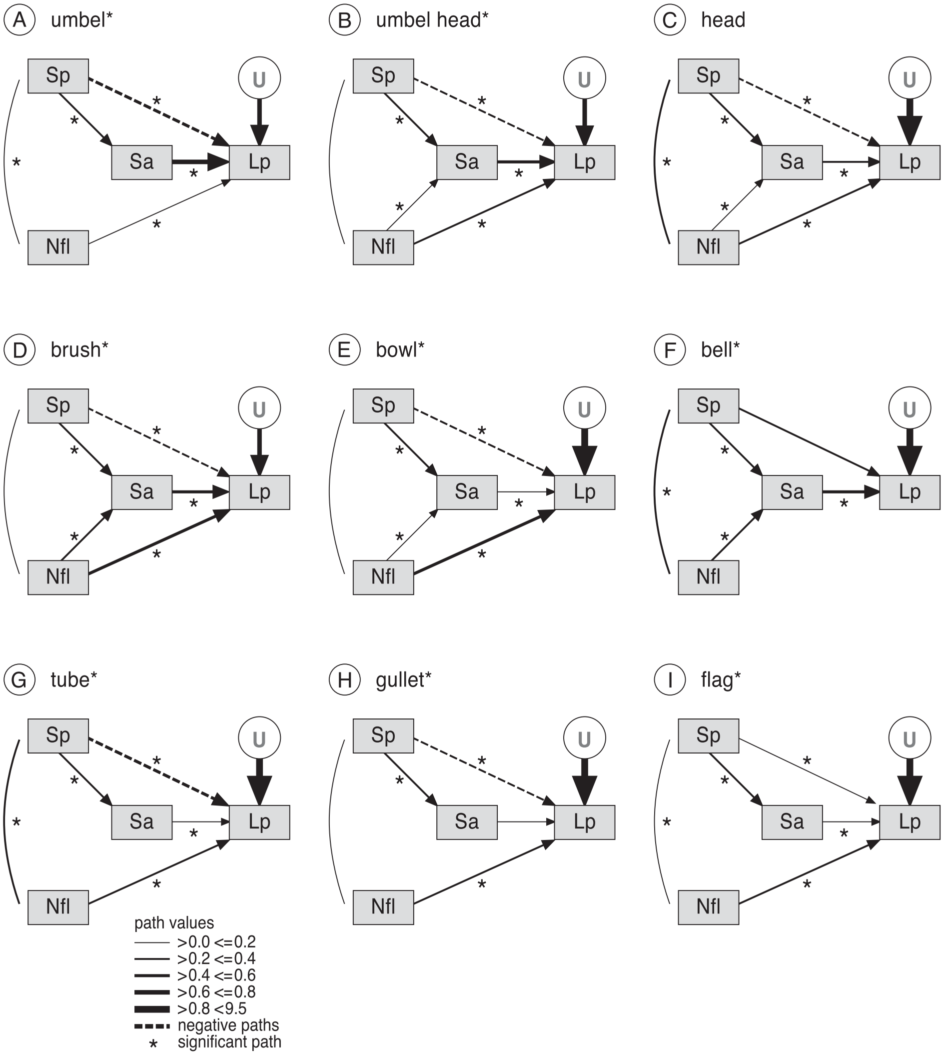


Fig. 4.3. Path models explaining variation in linkage level of the nine flower types (see text). Flower types are arranged in order of decreasing linkage level (table 4.2). Paths are indicated with arrows, correlations with lines. Thickness of lines corresponds with path values. Models with p>0.05 are marked with *. Sp= plant species richness, Sa= insect ("animal") species richness, Lp= plant linkage level, Nfl= flower abundance within flower type, Ap= total flower abundance, U= residual (unexplained) variation.

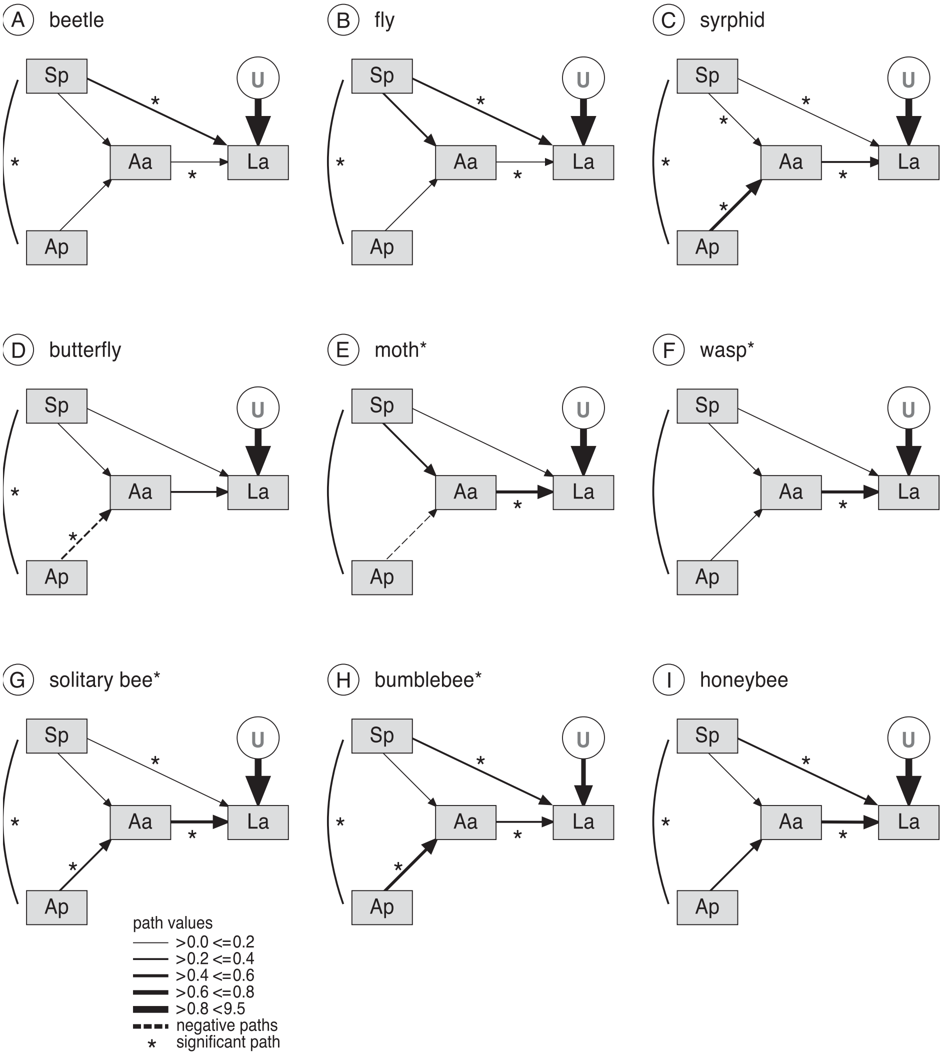


Fig. 4.4. Path models explaining variation in linkage level of the nine insect groups (in taxonomical order). Paths are indicated with arrows, correlations with unheaded lines. Thickness of lines corresponds with path values. Models with p>0.05 are marked with *. Sp= plant species richness, Sa= insect (“animal”) species richness, La= insect linkage level, Aa= insect abundance within group, Ap= total flower abundance, U= residual (unexplained) variation.

Table. 4.3. Insect linkage levels (mean and total L_a) of insect groups, and direct, indirect and total effects of the path analyses (models fig. 4.4). Since L_a of ants did hardly vary, no path model was analysed. The total number of censuses with insect observations was 322.

flower type	mean L_a ±SE[1] (N censuses)	total L_a ±SE[2] (N plant species)	effects			
				direct	indirect	total
beetle	1.24 ±0.05	10.5 ±4.1	S_p[3]	0.292	0.024	0.316
	(138)	(4)	A_a[4]	0.170	-	0.170
			A_p[5]	0	0.009	0.009
fly	1.46 ±0.03	15.6 ±2.9	S_p	0.379	0.018	0.397
	(278)	(17)	A_a	0.138	-	0.138
			A_p	0	0.011	0.011
syrphid	1.31 ±0.02	20.2 ±2.1	S_p	0.250	0.136	0.386
	(279)	(20)	A_a	0.382	-	0.382
			A_p	0	0.177	0.177
butterfly	1.17 ±0.03	14.38 ±2.1	S_p	0.049	0.030	0.078
	(159)	(8)	A_a	0.226	-	0.226
			A_p	0	0.004	0.004
moth	1.10 ±0.04	18	S_p	0.046	0.093	0.139
	(58)	(1)	A_a	0.481	-	0.481
			A_p	0	0.012	0.012
wasp	1.01 ±0.01	4.8 ±1.1	S_p	0.058	0.003	0.061
	(118)	(3)	A_a	0.541	-	0.541
			A_p	0	0.043	0.043
ant	1.10 ±0.04	16	-	-	-	-
	(51)	(1)				
solitary bee	1.17 ±0.03	6.9 ±1.2	S_p	0.183	0.044	0.227
	(139)	(8)	A_a	0.426	-	0.426
			A_p	0	0.101	0.101
bumblebee	1.43 ±0.04	26.5 ±7.5	S_p	0.303	0.009	0.312
	(219)	(6)	A_a	0.370	-	0.370
			A_p	0	0.098	0.098
honeybee6	1.42 ±0.07	33	S_p	0.354	0.014	0.368
	(103)	(1)	A_a	0.426	-	0.426
			A_p	0	0.080	0.080

[1] The mean of linkage levels over all censuses. [2] The mean over the total of the number of plant species an insect species visited during the whole research period (only insect species with >24 individuals). [3] Plant species richness. [4] Insect abundance per insect species. [5] Flower abundance. [6] The honeybee (*Apis mellifera*) is only one species.

DISCUSSION

Connectance and overall linkage levels

Our study showed that human-induced differences in species richness of plants and insects do indeed affect network parameters of communities of flowering plants and flower visiting insects. This was most obvious for connectance and plant linkage level.

Connectance (the ratio of observed plant-insect species interactions relative to all possible ones) was lower as plant species richness was higher: it could be observed in course of the season and across sites differing in plant species richness. Species richness of plants and insects increased in the course of the summer, but insects increased almost twice as much as plants. Therefore, the number of potential interactions between plant and insect species increased, too. The actual number of interactions increased less steeply, and therefore connectance decreased. Connectance was negatively related to plant species richness, but hardly related to insect species richness in the path analysis. This may be due to the fact that insect diversity was less different between the sites than plant diversity (chapter 3), and that flower-visiting insect species richness is strongly related to plant species richness. Jordano (1987) and Olesen & Jordano (2002) analysed connectance in relation to total species richness, and did not separate between plants and insects. They found a negative relation between connectance and total species richness, similar to the relation between connectance and plant species richness in our study. The mode (0.33) and mean (0.21) connectance from our study are well in concordance with other systems from temperate regions with less than 100 plant and insect species in total (Olesen & Jordano 2002); all our censuses had less than 60 plant and insect species. The high connectance in our system may indicate that the average site is relatively species poor (plants and insects), however, from the paper of Olesen & Jordano (2002) neither the area size nor the time span of the studies that they compared can be deduced. Therefore it is difficult to say how well our system fits into a general pattern. Stang *et al*. (2005) found that connectance is strongly affected by size constraints of nectar holders (depth and length of flowers) and insect mouth parts: this reduced the number of possible interactions by 57%! If this constraint is approximately the same in all communities, it would mean that, roughly estimated, connectance is always lower than 0.5 in plant-pollinator food webs. In our study, nearly all connectances were equal to or lower than 0.50 (apart from the 6 out of 331 censuses with only one plant species where connectance was 1.0 and one census with 0.58). Based on a scale between 0.0 and 0.5, the average connectance of 0.33 may indeed be high.

Plant linkage level was approximately two times higher than insect linkage level (total and mean per census). This is not surprising: as there are more insect species than plant species, plants should have more interactions (Dicks *et al*. 2002). Abundance of both plants and flowers explained a large part of the variation of linkage level. The correlation between abundance and linkage level was also observed in northern Sweden (Elberling & Olesen 1999). Linkage level may be underestimated, meaning that the more individuals of a species (plant or animal) are observed, the more interactions will be found. On the other hand, species that have higher linkage levels or that are more generalised may also be more abundant because of their generalism (Dupont *et al*. 2003; Vázquez & Aizen 2003). Despite the abundance effect, diversity was strong enough as a factor to be detected with path analysis and multiple regression.

Plant species diversity had opposing effects on plant linkage level: on the one hand, linkage level decreased as there were more plant species. But on the other hand, there was a positive effect of insect species richness on plant linkage level that in turn was positively affected by plant species richness. The (weak) net effect, however, is still negative, indicating that plants have fewer interactions as plant diversity increases.

Contrary to plant linkage level and the expectation that insect linkage level would also be negatively affected by species richness, it hardly varied. The effect of plant species richness was positive, contradicting findings of other studies (Heithaus 1974, 1979; Moldenke 1975). The separate analyses within insect groups, as proposed by Olesen & Jordano (2002), showed that these groups behave differently (see below). The same holds for the different flower types. A good reason for treating the groups separately is the skewness of the distribution of interactions: the minority of species interact with many other species from the other trophic level, especially among plants, whereas the majority have only few interactions (Jordano 1987; Ellis & Ellis-Adam 1993; Memmott 1999).

Flower types

The level of specialisation measured as linkage level varied much between the plant species: between 0.3 and 7.2 visiting insect species per census, or between 8 and 70 visiting insect species in total (table 4.2). The most specialised species had the flower types gullet, e.g. *Glechoma hederacea* (Lamiaceae) and *Rhinanthus angustifolius* (Scrophulariaceae) and flag, e.g. *Trifolium pratense* and *Lotus corniculatus* (Fabaceae). The most generalised plants had the flower type umbel (all in the Apiaceae family, e.g. *Anthriscus sylvestris* and *Heracleum sphondylium*). Stang *et al*. (2005) showed that nectar holder sizes (depth and width of flowers) and the number of flowers per plant explain 71% of the variation in the number of visitor species per plant species in southern Spain.

The Apiacae may be classified as "super generalists" (Olesen *et al*. 2002), because their mean and total linkage levels were between three and ten times larger than of the other flower types (table 4.2). Although insect species richness was the best explaining variable in the path model for umbels, the presence of the umbelliferous flower itself was already a cause for a high insect species richness (almost 20 percent of all insect species were exclusively observed on Apiaceae). Often on a single umbel or plant between 5 and 15 insect species could be observed during a census. More umbels or plants at a site added only few extra insect species and interactions, a possible explanation for why there was no path from flower abundance to insect species richness.

For all flower types except bell, plant species richness had a negative total effect on linkage level. This was strongest for the most specialised plant species (tube, gullet and flag). If more plant species are available, insects will choose plant species with more easily accessible flowers. Furthermore, insect species richness has a much smaller effect on linkage level, and therefore also on the indirect effect of plant species richness. In plant-flower-visitor networks, specialised species interact mostly with generalised species (Ellis

& Ellis-Adam 1993; Waser *et al*. 1996; Memmott 1999; Corbet 2000b); a measure of this is nestedness (Bascompte *et al*. 2003; Dupont *et al*. 2003). Although we did not analyse nestedness, we observed that the plant species with the lowest total linkage levels (thus the most specialised) were mainly visited by bumblebees and the syrphid fly Rhingia campestris, insects that are generalist at species level, although they may show temporal specialisation (flower constancy) at the individual level.

The flower type bell consisted of only one species (*Campanula rotundifolia*), and occurred at only five sites, which may explain why for the flower type bell a model different from that for the other flower types gave the best explanation for linkage level. It is the only species where plant species richness had a positive direct and total effect on linkage level. *Campanula rotundifolia* may be facilitated by other plant species in a community as *C. rotundifolia* will be visited by more visitor species in the presence of those other plant species. But to understand whether these extra species are also good pollinators, visitation rate (number of visits a flower head gets per unit time) and flower constancy, i.e. individual insect behaviour, have to be studied, along with pollen deposition and seed set (Kwak *et al*. 1996; Memmott 1999; Kwak *et al*. 2000; Thomson & Chittka 2001; Vázquez & Aizen 2003). *Campanula rotundifolia* was among others visited by oligolectic solitary bees specialised on Campanulaceae. Oligolectic bees may be more efficient pollinators than other species (Cane & Payne 1988; Corbet 1997; Blionis & Vokou 2001). When the best pollinator is absent, the plant species must rely on other visitors. The number and abundance of specialised bee species in our study area was significantly reduced at sites where land use intensity and fragmentation were high (chapter 3). This means that species with specialised interactions will be more vulnerable to loss of pollinators than species with generalised interactions (Rathke & Jules 1993; Bronstein 1995; Kwak *et al*. 1998; Johnson & Steiner 2000).

Insect groups

Flies, bumblebees, syrphids and the honey bee were the insect groups with the highest total and mean linkage levels. We may consider them as the most generalised. These groups had linkage levels that were between two and five times higher than those of the most specialised (lowest linkage levels) species: solitary bees, wasps and ants. The effect of diversity on linkage level differs between groups: whereas the most generalised groups tend to visit more plant species as there are more available, this is less the case for the other groups. Wasps were mostly observed on umbels, flowers with easily accessible nectar. Even though they have low linkage levels, their flower preference or specialisation is likely to be passive, as they do not have the physical ability to reach many flowers. Solitary bees are a group of species including generalist (polylectic) species and obligate specialist (oligolectic) species. These bee species are more actively specialised than wasps. Solitary bees mostly have flight periods of around six weeks (Westrich 1990) with a high coincidence of flight time and flower phenology of preferred food plants, particularly the oligolectic species. Bumblebees and honeybees are social insects that have to provide a nest with nectar and pollen during a whole season, and therefore must forage on many plant species.

Butterflies mostly occurred in low abundances and had a low mean linkage level which was as high as that of solitary bees (1.17), whereas the total linkage level of butterflies (14.4) is two times larger than that of solitary bees (6.9). Therefore it is likely that the butterflies' low linkage level can be explained by their low abundance: butterflies were observed in very low numbers within censuses, explaining a low linkage level. However, the total linkage level is based on the number of interactions of all individuals during the research period, and therefore gives a better estimate than the mean linkage level. The other species with low linkage levels (ants, wasps) are likely to be passive specialists: they simply cannot reach certain flowers, or they only visit flowers occasionally as they also use other food sources. Similarly, there are plant species with flower types that are accessible to all insects (like umbels) and others that are limiting the number of possible visitors, e.g. because they are too deep (like tube and several gullet flowers) or have to be forced open (like the flag-type flowers of the Fabacae).

Conclusions

We have shown that differences in species richness do affect connectance and linkage levels of plant-flower-visitor communities. This is similar to what was observed across geographical ranges and at larger temporal scales, but contrary to many of these, the effects differ between plants and flower visiting insects. We have also shown that the effects vary in strength and direction between taxonomical and functional groups of plants and insects. Because pollination of plants will depend on community interactions, a decrease in species richness may have negative consequences for pollination. Experimental research is needed to elucidate this for individual plant species.

ACKNOWLEDGEMENTS

We would like to thank Maaike de Vlas and Tineke Schwab for assistance in the field, and Theo Peeters, Wouter van Steenis, Kim Meijer, Kobus Boeke and Riek van Noordwijk, and late Henny Wiering for helping with the identifiction the insect species. This study was funded by the Stimulation Program Biodiversity of the Netherlands Organization for Scientific Research- Earth and Life Sciences (NWO-ALW).

5

Plant neighbourhood effects on plant linkage level, visitation rate and pollination

Frank Hoffmann and Manja M. Kwak

SUMMARY

This chapter addresses the questions: What is the effect of a plant species' neighbours on its visitation and pollination, and does the type of neighbourhood matter? A field survey and an experiment were performed to study this. For the field survey, the diversity and abundance of flowering plants and flower-visiting insects were investigated in road verges and ditch banks in the Netherlands in 2000 and 2001. From the data set six plant species with different visitor compositions were used for analysis: *Anthriscus sylvestris, Heracleum sphondylium, Angelica sylvestris, Tanacetum vulgare, Hypochaeris radicata* and *Hieracium laevigatum*. The effects of flowering plant species richness and community composition on plant linkage level (the number of insect species a plant species is visited by) were analysed. The type of other flowering species in a community rather than plant species richness affected plant linkage level. Plants that are more similar interact more with each other. The experiment was conducted with *Scabiosa columbaria*. Patches of potted *Scabiosa* plants were put into a flower-rich ornamental garden, a flower-poor grassland and into a maize field. The effects of plant neighbourhood on visitation rate, pollination and heterospecific pollen deposition were analysed. Pollen deposition was always lowest in the maize field. The percentage of deposited *S. columbaria* pollen relative to heterospecific pollen was lower than 50% in all patches. Potential seed set was always lowest in the maize field (between 5 and 40% of the flowers on a head would produce seeds), the grassland patch was close to 100% of flowers within a head. In conclusion, neighbourhood effects on visitors are plant species specific, and depend on the type of neighbouring species rather than total plant diversity. The number of visiting species and flower visitation rate alone may be misleading when one wants to evaluate facilitation or competition effects. The resulting pollination and reproduction may be different, even opposite. The balance between facilitation and competition depends on plant population size and flower density of both the target and other plant species in the community, and on plant community species composition.

INTRODUCTION

Biotic pollination is an important process by which plants interact. The interactions can be competitive or facilitative, and both will most likely occur between plant species with a shared pollinator fauna (Waser 1978b, 1979; Rathke 1983; Feinsinger 1987). Competition between plant species acts via the number of visitors, visitation rate, heterospecific pollen deposition and pollen loss, resulting in reduced seed set (Waser 1978a & b;Campbell 1985b; Campbell & Motten 1985; Armbuster & McGuire 1991; Jennersten & Kwak 1991; Kwak *et al*. 1998; Brown *et al*. 2002) or reduced pollen flow distance (Campbell 1985a). Facilitation can occur when a plant community as a whole attracts a relatively large number of flower visiting insects (Rathcke1983; Bäckman & Tiainen 2002; Collinge *et al*. 2003; Potts *et al*. 2003; chapter 3), thereby increasing the chance for appropriate pollinators (Corbet 1997). Furthermore, the presence of other plant species can be facilitating by increasing the visitation rate, i.e. the number of visits per unit time (Thomson 1978; Schemske 1981; Kwak 1988; Laverty 1991). The balance between competition and facilitation depends on the plant species, community composition and flower abundance and density (Rathcke 1983; Kwak *et al*. 1998).

The number of animal species that visit a plant species at a certain time and place is called plant linkage level (Jordano 1987; Olesen & Jordano 2002; Dupont *et al*. 2003). Plant species richness can affect plant linkage level negatively and positively (chapter 4). On one hand, as there are more plant species, insects may be distributed over more plant species, thus the number of insect species per plant species, or plant linkage level, will decrease. On the other hand, the number of insect species is positively affected by the number of plant species, which has a positive effect on plant linkage level. The strength of the effects depends on the flower type and specialisation level of a plant species, and on flower abundance and density. From the insect perspective, insect species tend to visit more plant species when more plants are available, but this relation is weak and differs significantly between insect taxa (chapter 4).

The ultimate causes for the pollination of plants are the choices made by individual flower visitors (Kwak *et al*. 1998). Flower constancy of individual visitors is affected by the availability of rewards (Goulson 1999; Hill *et al*. 2001) and by the composition of a flower community (Waser 1986; Chittka *et al*. 1997; Slaa & Biesmeijer 2003): flower constancy will be lower when insects perceive flowers of different plant species to be more similar. A low flower constancy can cause pollen loss to other plant species and deposition of heterospecific pollen on the stigmas of the target species (Rathcke 1983; Campbell 1985a). Heterospecific pollen deposition may not be caused by the diversity of a plant community per se, but by the densities and abundances of the component plant species in that community (Feinsinger *et al*. 1986). The effects of heterospecific pollen deposition on seed set are reported to be neutral (Schemske 1981; Campbell & Motten 1985; Kwak & Jennersten 1986 Kwak & Bergman 1996) or negative (Waser 1978b; Randall & Hilu 1990; Brown & Mitchell 2001).

In this study we investigated effects of flower species richness and community composition on plant linkage level of six plant species: *Anthriscus sylvestris, Heracleum spho-*

ndylium, Angelica sylvestris, Tanacetum vulgare, Hypochaeris radicata and *Hieracium laevigatum*. In addition, we conducted an experiment to analyse the effects of flower species richness on visitation rate, pollination and heterospecific pollen deposition for *Scabiosa columbaria* (Dipsacaceae). This plant species is very appropriate for studying pollination for various reasons.

MATERIAL AND METHODS

Field study with six plant species

We conducted our study in 2000 and 2001 in the province of Drenthe in the north of the Netherlands (ca. 53°00' N, 6°35' E). We inspected ca. 325 km of road verges and ditch banks to select 53 linear sites of 100 m length and 1-2 m width. The sites were not shaded. Verges along large roads and motorways were avoided for safety reasons. The field methods for obtaining the data of insect species visiting flowers are described in chapter 2 and 3.

From the data set we selected plant species from various plant families and with different flower types (chapter 2). For the analyses, each selected plant species should have a series of sites within one cohort. Per plant species only one cohort was selected to prevent seasonal effects on visitation. A plant species should at least have 10 individual insect visitors per site, and there should be at least 10 sites within one cohort. Six plant species from two plant families met the criteria, from the Apiaceae: *Anthriscus sylvestris*, *Heracleum sphondylium* and *Angelica sylvestris*, and from the Asteraceae: *Tanacetum vulgare*, *Hypochaeris radicata*, and *Hieracium laevigatum* (table 5.1). As all six species have relatively high linkage levels (table 5.1) and have flowers that are accessible to a large number of insects, they can be considered to be generalist species (chapter 2). The specialist plant species in the data set did not fit the criterion of a minimum of 10 sites with 10 visitors per site.

Table. 5.1. Linkage levels (L_p) of the six selected plant species for the whole research period and the selected cohorts (see Material and Methods for selection criteria).

	whole research period 2000-2001				selected cohort in 2000		
target species	N sites	N censuses	total Lp[1]	mean Lp[2]	period	N sites	mean Lp ±SE
Anthriscus sylvestris	45	124	123	8.9	27 May	22	8.9 ±0.7
Heracleum sphondylium	29	90	122	8.7	23 July	13	11.9 ±1.4
Angelica sylvestris	15	38	107	12.7	12 August	13	14.1 ±1.6
Tanacetum vulgare	19	52	85	7.8	12 August	11	14.5 ±1.2
Hypochaeris radicata	34	132	95	4.4	19 June	12	8.3 ±1.2
Hieracium laevigatum	30	123	107	4.9	5 July	11	10.1 ±1.7

[1] The total number of visiting insect species during the research period. [2] The mean linkage level per census.

For the six plant species, plant linkage levels (L_p) were calculated. The total linkage level is the total number of insect species that were observed on a plant species during the whole research period. Also two mean linkage levels were calculated: the mean number of insect species over all censuses in 2000 and 2001 (the mean L_p over the whole research period, table 1), and the mean number of insect species over the sites in the selected cohorts (the mean L_p over the selected cohort).

We analysed the effect of plant species richness and of different flowering plant community components on plant linkage level (per cohort). Therefore the abundances of the plant species were divided into groups: (1) the target plant species itself, (2) generalist neighbouring species, i.e. species with accessible nectar (actinomorph flowers), and (3) neighbouring specialist species, i.e. flowers with deep or hidden nectar (zygomorph and tubular flowers). Within the generalists we also wanted to distinguish the most closely related plant species, with similar flowers as the target species, from less related species. For *Anthriscus sylvestris*, *Heracleum sphondylium* and *Angelica sylvestris* this would have been Apiaceae species, but the number of flowers of related plant species flowering simultaneously with the target species was too low to distinguish a separate group. For *Hypochaeris radicata* and *Hieracium laevigatum* these were yellow Asteraceae: species from the genera *Hieracium, Hypochaeris, Leontodon, Crepis* and *Lapsana*. For *Hypochaeris radicata* and *Hieracium laevigatum* "generalists" did not include neighbouring yellow Asteraceae.

Experiment with *Scabiosa columbaria* L. (Dipsacaceae)

Scabiosa columbaria is a gynodioeceous, protandrous perennial of dry calcareous grasslands (Ouborg *et al*. 1991; Kwak 1993; van Treuren *et al*. 1993; Velterop 2000). The hermaphroditic flowers are arranged in heads of 40-100 flowers, each containing one ovule. Heads start flowering in the male phase for five to seven days, during which new flowers open continuously. After all flowers have opened, the whole head enters the female phase, usually lasting for only a single day. *Scabiosa columbaria* is dependent on insects for pollination, the main pollinators are syrphid flies, bumblebees and solitary bees (Kwak 1993; Velterop 2000).

The site where the experiments were conducted was situated in a private garden near the city of Assen (52°59'N, 6°35'E), within the same region as the transect observations. On two days, 11 September and 2 October 2000, we constructed three artificial patches of potted *S. columbaria* plants to investigate the effects of plant neighbourhood diversity on visitation and pollination. Each patch had a different neighbourhood: (1) a species- and flower-rich ornamental garden, (2) a species- and flower-poor meadow, and (3) a maize (*Zea mays*) field. The maize was >1.80 m tall, therefore the patch was hardly visible. The patches and neighbourhoods were approx. 50 m apart. A patch consisted of 28 flower heads in the male phase and 7 flower heads in the female phase. In total each patch consisted of 35 flower heads. The male heads were on the potted plants, the female heads were kept in glass tubes filled with water attached to a stick. The female heads were collected in the morning prior to the experiment and were virgin at the start of the experiment. This was checked with a hand magnifier.

The patches were set up around 11:00 h and removed at 15:00 h. Between 13:00 and 14:30 h, six 10 minute period observations of visitation rate were done, simultaneously at each patch. We did not immediately start with the observations after installation of the patches, because we wanted the insects to discover the patches first. Between each 10 minute period the observers rotated between the patches. The number of visits was scored per insect group, i.e. houseflies (*Musca* spec.), *Eristalis tenax*, other *Eristalis* species, *Helophilus* spec., other syrphids, butterflies, bumblebees, honeybees, and "other". From this was calculated the visitation rate (number of visits per flower head per 10 minutes) and the proportion of visits per insect species.

After the experiment the female flower heads were collected. For each head, on 15 stigmas the number of deposited *S. columbaria* pollen grains was counted using a 15x hand magnifier. From the distribution of pollen grains over the stigmas the potential seed set was calculated, based on a minimum of four pollen grains per stigma needed for fertilisation (Velterop 2000). Then per flower head all the stigmas were cleaned using a piece of sticky gel (Beattie 1972), from which a microscope slide was made by melting the gel. The sticky gel contains the colouring agent fuchsin that colours plant tissues red, allowing the identification of pollen grains under a microscope. *Scabiosa columbaria* and heterospecific pollen grains were counted with a maximum of 300 grains per sample. All grains were counted when there were less than 300 grains in a sample. The percentage of conspecific pollen (*S. columbaria*) was calculated.

Statistics

Pearson correlations were used for finding correlations between plant linkage level and plant species richness the plant groups. For the analyses the absolute and the relative number of flowers at a site were used. The treatment effects in the experiment were analysed with Kruskal-Wallis tests, and a Mann-Whitney procedure for differences between groups (Zar 1984). All analyses were performed with the package SPSS for Windows 12.0.1 (2003).

RESULTS

Field study with six plant species

The total linkage levels varied between 85 and 123 visitor species, and the mean linkage levels between 4.9 and 12.7 visitor species, both over the whole research period (table 5.1). *Anthriscus sylvestris* and *Heracleum sphondylium* had the highest total linkage levels, *Angelica sylvestris* the highest mean linkage levels. *Tanacetum vulgare* and *Hypochaeris radicata* had the lowest total linkage levels, and *H. radicata* and *Hieracium laevigatum* the lowest mean linkage levels. The mean linkage levels over the selected cohorts varied between 8.3. and 14.5. The mean plant linkage levels over the selected cohorts were higher than over the whole research period for all species, except for *Anthriscus sylvestris* (table 5.1).

Plant linkage level was not significantly correlated with flowering plant species richness for any of the six selected plant species (table 5.2). Linkage level was positively correlated with flower abundance of the target species for *H. radicata* and *H. laevigatum, T. vulgare* and *H. sphondylium* (table 5.2, fig. 5.1).

The relative flower abundance expresses the number of flowers of the different plant groups (target species, generalist and specialist species or yellow Asteraceae) relative to the total number of flowers. Plant linkage level was positively correlated with the relative abundance of the target species for *H. radicata* and *H. laevigatum* (table 5.2, fig. 5.1).

For two plant species correlations were found with other plants groups: For *H. radicata*, the relative number of yellow Asteraceae flowers was negatively correlated with plant linkage level (fig. 5.2a, table 5.2). For *Angelica sylvestris*, the absolute number of generalist flowers was positively correlated with linkage level (fig. 5.2b, table 5.2). Correlations were found neither between plant linkage level and specialist flowers, nor between any variable and linkage level of *Anthriscus sylvestris*.

Experiment with *Scabiosa columbaria*

Visitation rate

Flower visitation was strongly influenced by the type of neighbourhood. Visitation rate (fig. 5.3A) was lowest at the patch in the maize field on 11 September (X^2=12.7, df= 2, p< 0.005) and 2 October (X^2=11.6, df= 2, p< 0.005). On both days, the ornamental garden patch tended to have a higher visitation rate than the meadow patch (not significant). The patch in the maize field received 6-12 times less visits in total than the other two patches.

Table. 5.2. Correlations between plant linkage level and flower community composition variables, viz. plant species richness, flower abundance of the target species itself, abundance of generalist flowers, abundance of specialist flowers and abundance of yellow Asteraceae.

	Pearson correlation coefficients								
	Species richness[1]	target species		generalist[2]		specialist[3]		yellow Asteraceae	
target species (N sites)		abs[4]	%[5]	abs	%	abs	%	abs	%
Anthriscus sylvestris (22)	0.23	0.30	-0.03	0.02	-0.17	0.30	0.24	-	-
Heracleum sphondylium (13)	-0.31	0.69**	0.20	-0.43	-0.33	-0.11	0.16	-	-
Angelica sylvestris (13)	0.30	0.10	-0.23	0.57*	0.11	0.36	-0.10	-	-
Tanacetum vulgare (11)	-0.25	0.66*	0.06	-0.59	-0.24	0.24	0.39	-	-
Hypochaeris radicata (12)	0.25	0.72**	0.67*	0.09	-0.14	-0.4	-0.3	-0.42	-0.69*
Hieracium laevigatum (11)	0.48	0.86**	0.93**	0.12	-0.24	-0.42	-0.55	-0.15	-0.35

[1] The number of flowering plant species at a site. [2] All non-zygomorph species, except yellow Asteraceae for *H. radicata* and *H. leavigatum*. [3]All species with deep or hidden nectar (zygomorph and tubular flowers). [4]The absolute number of flowers at a site. [5]The relative number of flowers at a site. *Correlation significant at the 0.05 level. **Correlation significant at the 0.01 level.

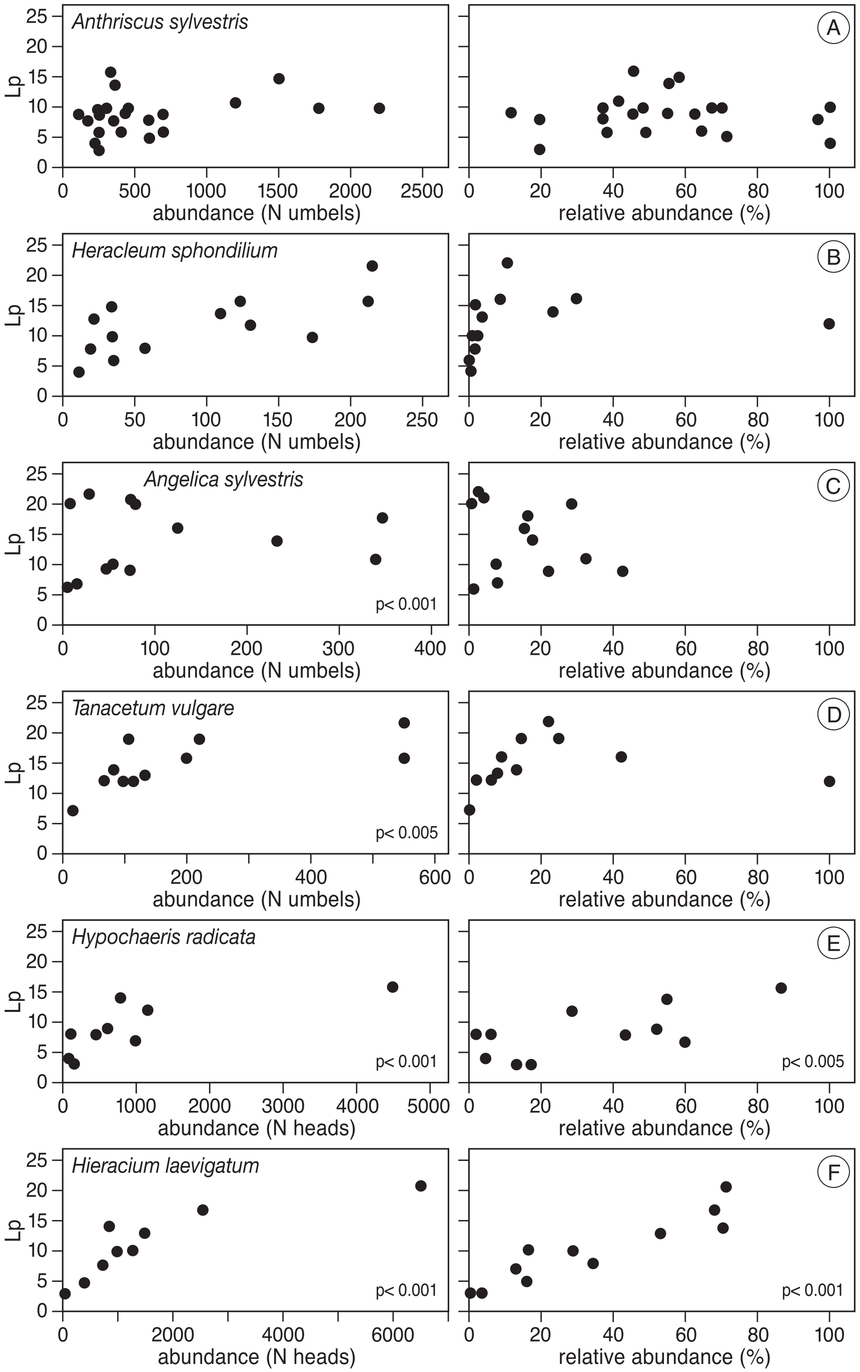


Fig. 5.1. Relation between flower abundance of the species in question (absolute: left, and relative: right) and plant linkage level (L_p) for six plant species. Correlation coefficients are listed in table 5.2.

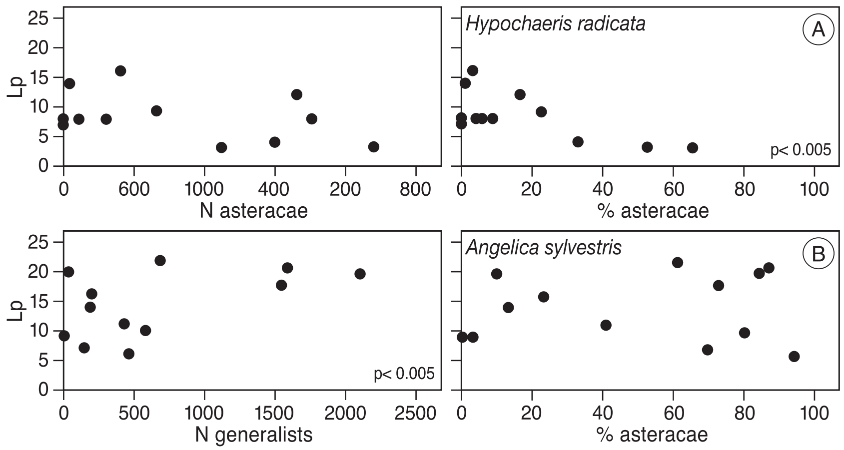


Fig. 5.2. Relation between flower community composition variables and plant linkage level (L_p). (A) *Hypochaeris radicata*: the absolute (left) and relative (right) number of other yellow Asteraceae flower heads at a site. The number of Asteraceae is without the abundance of *H. radicata*. (B) *Angelica sylvestris*: the absolute (left) and relative (right) number of other generalist flower units at a site. The number of generalists is without *A. sylvestris*. Correlation coefficients in table 2.

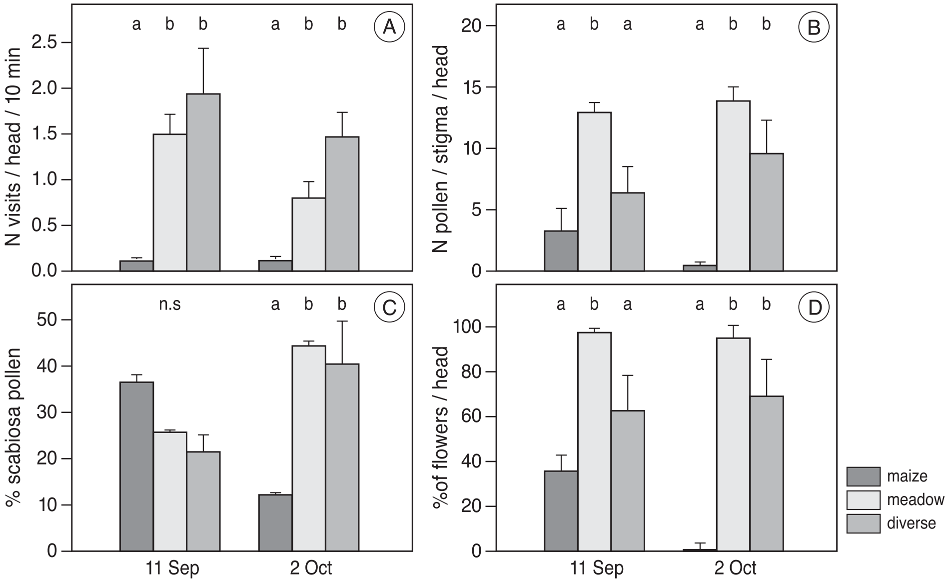


Fig. 5.3. Means ±SE of (A) visitation rate, (B), pollen deposition of *Scabiosa columbaria* pollen grains, (C) purity of stigmatic load (deposited pollen) and (D) potential seed set of introduced patches with potted plants of *Scabiosa columbaria*. Patches consisted of 28 male and 7 female flower heads, and were placed in the neighbourhood types diverse (flower rich garden), meadow (open grassland with few flowers) and maize (dense maize field). Female flower heads were virgin at the start of the experiment. Significances are tested with univariate analysis of variance. Different letters indicate significant differences (see text forreplicate numbers).

COMPOSITION OF VISITS

The number of visiting taxa can be regarded as a measure of plant linkage level (L_p). The number of visiting taxa varied between one and eight per patch per observation day (table 5.3). In the flower-rich ornamental garden, L_p was always highest: eight taxa were observed on both days (table 5.3), and always lowest in the isolated maize field (one or three taxa). The flower-poor meadow patch was intermediate, between seven and four taxa. The main visitors in the ornamental garden and the meadow were large syrphid flies, mainly *Eristalis tenax* and other *Eristalis* species (fig. 5.4). Most of the few visitors in the maize field were small syrphid flies (*Platycheirus* species).

Table. 5.3. Linkage levels (L_p) in the introduced patches of *Scabiosa columbaria* on two days in 2000. Patches were placed in the neighbourhood types diverse (flower rich garden), meadow (open grassland with few flowers) and maize (dense maize field). Linkage level is the number of taxa observed during the plot observations (see text and fig. 5.4 for the taxa).

patch type	11 September	2 October
diverse	8	8
meadow	7	4
garden	3	1

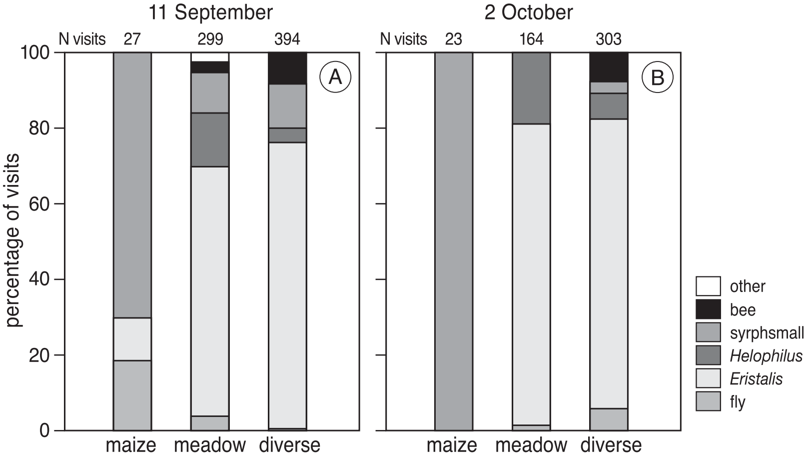


Fig. 5.4. Composition of visits in introduced patches with potted plants of Scabiosa columbaria on 11 September (A) and 2 October (B). N visits are the total number of visits brought during an observation period. Patches consisted of 28 male and 7 female flower heads, and were placed in the neighbourhood types diverse (flower rich garden), meadow (open grassland with few flowers) and maize (dense maize field). Female flower heads were virgin at the start of the experiment. The visitors are (1) Diptera: fly= *Musca* species, Eristalis= *Eristalis tenax*, *E. arbustorum* and *E. horticola* (syrphids), helophilus= *Helophilus pendulus* and *H. trivittatus* (syrphids), and syrphsmall= other small syrphids, (2) Hymenoptera: bee= bumblebees (mainly *Bombus pascuorum*) and honeybees (*Apis mellifera*), and (3) other insects.

POLLEN DEPOSITION

Pollen deposition (fig. 5.3B) was higher in the meadow patch than the other two patches on 11 September ($X^2 = 9.1$, df= 2, $p < 0.05$), and on 2 October, it was higher in the ornamental garden and meadow than in the maize field ($X^2 = 11.1$, df=2, $p < 0.005$). The percentage of deposited *S. columbaria* pollen relative to heterospecific pollen was lower than 50% in all patches (fig 5.3C). On 11 September, there was no significant difference, on 2 October the maize field was lowest, around 10% ($X^2 = 9.1$, df= 2, $p < 0.05$). The meadow patch tended to have a marginally higher proportion of *S. columbaria* pollen than the ornamental garden patch. Potential seed set (fig. 5.3D) was always lowest in the maize field (between 5 and 40% of the flowers on a head would produce flowers), in the meadow patch it was close to 100%. In the ornamental garden it was intermediate on both days (60- 70%). On 11 September, the meadow patch was higher than the other two ($X^2 = 11.2$, df= 2, $p < 0.005$). The difference between the meadow and the maize patches was only significant on 2 October ($X^2 = 13.3$, df= 2, $p < 0.005$).

DISCUSSION

Field study

In the field study, the type of other flowering species in a community rather than plant species richness affected plant linkage level. Contrary to what was observed in chapter 4, species richness was not correlated with plant linkage level (the number of visiting insect species). Analysing six individual species may be the reason: in chapter 4, whole communities or functional groups of species were analysed. This means that patterns observed at community level will not necessarily be observed at species level, at least not for all species. For *Hypochaeris radicata*, the relative abundance of yellow Asteraceae, thus related plant species with similar flowers, was negatively correlated with linkage level. It indicates that related Asteraceae species may compete for pollinators, and that the outcome depends on their relative abundances. The competition will depend on the individual behaviour of insects, which is partly determined by the similarity between plant species (Waser 1986; Chittka *et al*. 1997; Hill *et al*. 2001; Slaa & Biesmeijer 2003). Although many yellow Asteraceae species are not easy to distinguish for (unexperienced) people, insects may see the difference between several species. This can be related to reward availability (Torres & Galetto 2002) or to UV-radiation reflection (van der Muren *et al*. 2003).

Flower abundance had the highest correlation with plant linkage level in four species with only one species in the Apiaceae family (*Heracleum sphondylium*). This is in concordance with chapter 4, where linkage level was strongly related to flower abundance for all flower types (including Asteraceae), but not the Apiaceae. Often on a single umbel or plant between 5 and 15 insect species could be observed. Observing more umbels or plants at a site added only few extra insect species and interactions to an Apiaceae species (chapter 4). Visitation of *Anthriscus sylvestris* was not affected by any variable at all. It is a species flowering early in the season and has a very dominant position in the vegetation of road verges. There were only few generalist plant species present during its flowering period, and the visitor composition overlap with specialist plant species is very low (chapter 2). *Aegopodium podagraria* is the only Apiaceae species in our research area that had a phenology overlapping with the end of the flowering period of *Anthriscus sylvestris*. It was not enough for making Apiaceae a separate group in the analyses like the Asteraceae for *Hypochaeris radicata* and *Hieracium laevigatum*. For *Angelica sylvestris* there was a positive correlation between the abundance of generalist plant species and the number of visiting insect species. This can mean that the number of visitor species *Angelica sylvestris* is facilitated by other plant species. On the other hand, *Angelica* may be a good competitor, and draws away insects from other plant species. In our study area it is the plant species with the highest mean linkage level over all censuses (table 5.1). It differs from *H. sphondylium* by attracting more Hymenoptera, particularly wasps, but also Lepidoptera (chapter 2). Thus although Apiaceae may appear very similar at first (human) sight, the visitor guilds of the Apiaceae species can be rather different, like also stated for the Asteraceae above. Also (Ellis & Ellis-Adam 1994a) observed that "one umbel is not like the other". Whether Apiaceae species affect each other's visitor composition or compete for pollination cannot be concluded from our data. Dlussky (1998) found that *Anthriscus sylvestris* and *Aegopodium podagria* affect each other's pollinator composition when they co-occur.

The presence of specialised plants (zygomorph flowers or flowers with hidden nectar) was not correlated with linkage level of any of the six species studied: They were all highly generalised. The specialised plant species could not be selected as target species themselves, because the number of observed visitors was too low to meet the criteria of ten sites with at least ten visitors each within one cohort. Analysing effects for these species requires another method than ours. For example, measuring visitation rates or counting individuals during a longer time of a day may increase the amount of data per site.

Experiment

In the experiment with *Scabiosa columbaria*, the "linkage level", i.e. the number of species from the visitation rate observations, was always highest in the patch in the ornamental garden, so with a neighbourhood where flower diversity and flower abundance are highest, too. Although the data are collected on two observation days, the results showed that a high visitor diversity and visitation rate is no guarantee for a high pollen deposition. Although the meadow patch had a lower visitation rate than the ornamental garden patch, the deposition of pollen and the potential seed set were higher. The visitation rate was highest where flower diversity was high, but flower constancy may be lower, resulting in heterospecific pollen deposition. Most visits were brought by syrphid flies in both patches. Syrphids can be flower constant for a restricted time period (Haslett 1989; Goulson & Wright 1997; Sutherland *et al*. 1999)

The purity of deposited pollen on the stigmas is the sum of the depositions by all the insects that visited a flower. The purity of deposited pollen hardly differed between the patches. The amount of heterospecific pollen on the grassland patch (>50%) showed that insects in that patch previously must have visited other plant species as well. Plants can compete at larger distances than the direct flower neighbourhood, depending on the insect's flight distances (Rathcke 1983; Osborne *et al*. 1999). In chapter 7 is shown that syrphids visiting *Succisa pratensis* can carry between 30 and 80% heterospecific pollen grains, including plant species not occurring in the direct vicinity. Although individual syrphid species may be constant for the time that they were observed, they may carry a mixed body pollen load. This load reflects the plant species that were visited during a longer period, preceding the foraging in a *S. pratensis* population.

Isolation and patch size are other factors regulating flower constancy and residence time of a flower visitor: as patches are more distant from other flowers, visitors tend to stay longer in a patch (Sowig 1989; Kwak *et al*. 1998; Wolf & Harridson 2001). The maize field patch was completely isolated, which was reflected by the low visitation rates, number of visitor species, pollen deposition and potential seed set. In the ornamental garden, insects may have changed more between the *S. columbaria* patch and the diverse flower neighbourhood, but within the meadow patch they stayed longer. In the meadow only a few scattered *Leontodon autumnalis* flowers were present. Therefore the lower pollen deposition in the garden patch can be due to pollen loss to the surrounding neighbourhood. So, at first sight, the diverse neighbourhood seemed to be facilitating at the level of visitation rate. But at the level that matters for reproduction, pollination, it was competing!

When a plant has obligate specialist visitors like oligolectic bees, they may "escape" from a competing neighbourhood. Strickler (1979) and Cane & Payne (1988) showed that oligolectic bees can have a better pollination quality than more generalist visitors. Also for *S. columbaria* this is the case (Velterop 2000). In the Netherlands, the specialists for *S. columbaria* are endangered and are restricted to the south of the country (Peeters *et al*. 1999). In our field study, several oligolectic bee species visited yellow Asteraceae, among others *Hypochaeris radicata* and *Hieracium laevigatum*. However, they seem to visit most of these plant species in the order they encounter them during a foraging trip (F. Hoffmann, pers. obs.): the bees are specialised at the level of plant family or genus, and not species. *Tanacetum vulgare* was visited by one monolectic bee species (*Colletes daviesanus*), but its numbers were extremely low compared to the abundance of the diverse array of other visitor species (chapter 2).

Perspectives and conclusions

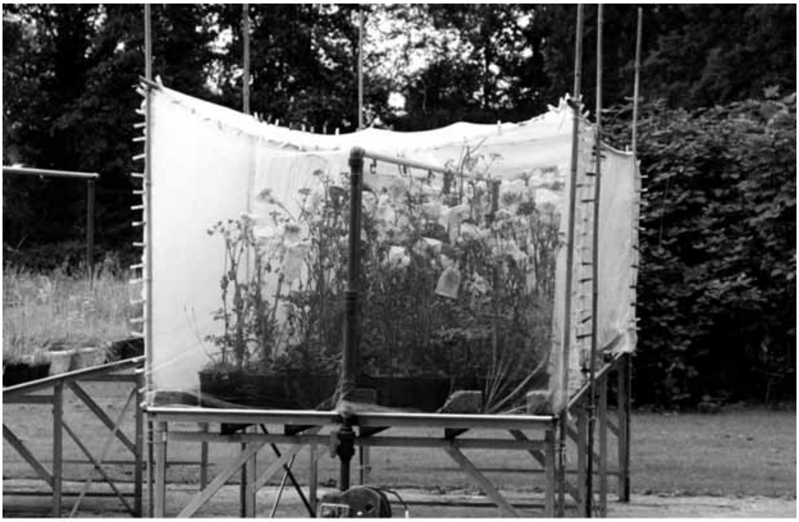
We have shown that neighbourhood effects on visitors are plant species specific, and depend on the type of neighbouring species rather than total plant diversity. The number of visiting species (plant linkage level) and flower visitation rate alone may be misleading when one wants to evaluate facilitation or competition effects: the resulting pollination and reproduction may be different, even opposite. The balance between facilitation and competition depends on plant population size and flower density of both the target and other plant species in the community, and on plant community species composition.

In both the field study and the experiment, neighbourhood had an effect on either visitation or pollination. However, the concept of neighbourhood was not the same in the two cases: in the field study, the neighbourhood was the flower community composition, and the target species were part of the flower community. In the experiment, an artificial patch of the target species was introduced in environments differing in species richness and patch visibility for insects.

The best way to study neighbourhood effects and test theories about competition and facilitation of visitation and pollination (Rathcke 1983; Feinsinger 1987; Goulson 1994; Westerkamp 1997; Campbell *et al*. 2002; Feldman *et al*. 2004) is to use controlled experiments. Patch sizes and flower densities of the target species can be kept constant with potted plants, whereas the type of neighbours can be varied. Experiments should be repeated with different flower densities and plant species mixtures of the neighbourhood, and should also include seed set measurements. Our study is an example of such an approach.

ACKNOWLEDGEMENTS

We would like to thank Meike Bulten and the students participating in the undergraduate General Ecology course in 2000 for helping to gather the data for the *Scabiosa columbaria* experiment.



6

The vulnerability to pollinator species loss of plant species with various pollination syndromes and degrees of specialisation

Frank Hoffmann and Manja M. Kwak

SUMMARY

This chapter addresses the problem whether pollinators of plant species with various pollination syndromes and degrees of specialisation can be replaced to compensate for a decrease of insect diversity. A field experiment was conducted with generalist plants with generalist visitors (*Anthriscus sylvestris* and *Succisa pratensis*), specialist plants with generalist visitors (*Phyteuma spicatum* subsp. *nigrum* and *Scrophularia nodosa*), and specialist plants with specialist visitors (*Campanula rotundifolia* and *Lysimachia vulgaris*). Patches of potted plants were placed in environments differing in land use and plant species composition. Insect visitation at the sites and the resulting seed set were measured. The following questions were asked: 1. How important is insect-pollination compared to self-pollination for the reproduction of the target species? 2. What are the effects of different environments on the insect visitor diversity, visitation rate and resulting seed set of the target plant species? 3. Do the target plant species have alternative pollinators? Seed set of the plant species with potential self-pollination capacity was considerably reduced without insect visitation. For both generalist and specialist plant species the composition of visits varied. Differences in visitation rate between environments were found for three specialist plant species, including both target species with specialist visitors. The effects of the environments on seed set were partially opposite compared to those on visitation rate for the plant species with generalist visitors. For *Campanula* and *Lysimachia* the absence of their oligolectic bees had a negative effect on visitation rate, seed set and germination. The results confirmed the hypothesis that specialist plant species with few specialist pollinators are the most vulnerable to pollinator loss. For very common and extremely generalist plant species, pollinators can be replaced, as there will always be some visitors present acting as pollinators. For the remaining generalist and specialist plant species with generalist pollinators it is currently difficult to predict the vulnerability to pollinator loss. This study has shown that even within larger studies, plant species will need an individual approach to elucidate the processes involved.

INTRODUCTION

Changes in land use and agricultural intensification cause habitat deterioration and fragmentation, resulting in species decline, reduction of population sizes and changes in the species composition of communities (Fahrig 2003). In agricultural landscapes, linear habitat fragments, such as road verges and ditch banks, often are the only natural habitat remnants. In the Netherlands for example, roadside habitats cover up to 2.1 percent of the total land area, which is a considerable amount compared to 4.2 percent of natural areas in Netherlands (Schaffers 2000). For organisms in such refugia not only the habitat and populations themselves are smaller, but the environment as such in the surrounding matrix is different compared to more continuous habitats (Rathcke & Jules 1993).

More than 70 percent of all angiosperms are insect-pollinated (Kearns & Inouye 1997). Changes in insect species richness and composition and changes in plant population size and structure can affect pollination (Rathcke & Jules 1993; Buchmann & Nabhan 1996; Kremen & Ricketts 2000). For many plant species a decline in pollination quantity (the number of visitor species per plant species, and the number of visits and pollinations) and quality (con- and heterospecific pollen deposition) will result in a lower seed set (Ågren 1996; Kwak *et al*. 1998; Steffan-Dewenter & Tscharntke 1999; Tomimatsu & Ohara 2002) and/ or inbreeding (van Treuren *et al*. 1993; Oostermeijer *et al*. 2000; Velterop 2000; Luijten 2001; Mustajärvi *et al*. 2001).

Plant species have different modes of pollination, characterised by different flower types, pollinator species and other features. These functional types are often referred to as pollination syndromes (Fægri & van der Pijl 1979; Armbuster *et al*. 2000; Ollerton & Watts 2000). Pollination syndromes describe "typical" systems (Fægri & van der Pijl 1979; Ollerton & Watts 2000), but are not applicable to all plant species (chapter 2), maybe because the majority of plant-pollinator interactions are of an opportunistic and generalist nature (Waser *et al*. 1996; Kearns *et al*. 1998; Memmott 1999; Armbuster *et al*. 2000; Johnson & Steiner 2000). The degree of ecological specialisation of plants and animals is continuous across species (Armbuster *et al*. 2000), and the match between the degree of specialisation of plants and their pollinators is distributed asymmetrically across species (Armbuster *et al*. 2000; Johnson & Steiner 2000): For example, generalist plant species can be visited by specialist insects, and specialist plant species by generalist insects. Insects, mainly bees, with an obligate specialisation for one or few related plant species as pollen source are called oligolectic, whereas species that have no specialisation are polylectic (Westrich 1990, 1996).

The generalised nature of pollination of many plant species may result in the resilience to pollinator species loss, because pollinator species that have disappeared may be replaced with other species (Waser *et al*. 1996; Spira 2001). However, it is unclear whether plant species with different degrees of specialisation have different possibilities for alternative pollinators. Plant species with just a few specialist pollinators are said to be the most vulnerable, as the loss of pollinator species may leave few or no alternatives (Rathcke & Jules 1993; Bronstein 1995; Kwak *et al*. 1998; Johnson & Steiner 2000). We are not aware of any concrete examples of the decline of such plant species caused by the lack of pollinators.

In this study, we are interested in how insect visitation, pollination and seed set change when the environment of a plant changes. A field experiment was conducted with six plant species with different pollination syndromes and degrees of specialisation: *Anthriscus sylvestris*, *Succisa pratensis*, *Phyteuma spicatum* subsp. *nigrum, Scrophularia nodosa*, *Campanula rotundifolia* and *Lysimachia vulgaris*. The plant species vary between generalists with many different unspecialised insect visitor species, and specialists with few, highly specialised insect visitors. We will address the following questions: (1) How important is insect pollination compared to self-pollination for the reproduction of the target plant species? (2) What are the effects of the environments on the insect community diversity, and the insect visitor diversity, visitation rate and resulting seed set of the target plant species? (3) Do the target plant species have alternative pollinators in the different environments? Patches of potted plants will be installed in road verges in environments differing in land use and plant species composition. Those environments are expected to affect the species composition and behaviour of flower-visiting insects, and in that way pollination and reproduction.

MATERIAL AND METHODS

Study area

We conducted our study in the Netherlands in the north of the province of Drenthe (ca. 53°00'N, 6°35' E), an area of Pleistocene origin. The experiments were conducted in road verges situated in environments with different types of land use (chapters 2 - 4). The greater part of the area is used for agricultural purposes and has undergone several rounds of land reallocations between the 1950s and 1990s. Part of the area is in or adjacent to natural reserves of semi-natural hay meadows along the streams of the Drentse Aa and Elperstroom. Land use varies between heavily fertilised and sprayed intensive grassland and arable land (potatoes, cereals, maize, sugar beet), and semi-natural hay meadows mown once or twice a year (mostly in natural reserves).

Plant species

The plant species selected for this experiment have different pollination syndromes and degrees of specialisation, occur in the research area, and can be grown in pots easily. Six target plant species were used: *Anthriscus sylvestris*, *Succisa pratensis*, *Phyteuma spicatum* subsp. *nigrum*, *Scrophularia nodosa*, *Campanula rotundifolia* and *Lysimachia vulgaris*, nomenclature follows van der Meijden *et al*. 1996; Tamis *et al*. 2004. The plant species will be named by their genus in the rest of this text.

Characteristics of the species are listed in table 6.1: taxonomy, regional and national red list status, reproductive and flower biology, phenology, and important flower visitors. The plant species can be grouped into three functional types: generalist plants with mainly generalist pollinators (*Anthriscus* and *Succisa*), specialists with mainly generalist pollinators (*Phyteuma* and *Scrophularia*) and specialists with mainly specialist pollinators (*Campanula* and *Lysimachia*). The generalist plant species have easily accessible flowers with many different visitor species, among which some also have other food sources than flower products, especially on *Anthriscus* (chapter 4). The specialist plant species have flowers that are difficult to access or recognize due to flower morphology (*Phyteuma*, *Scrophularia* and *Campanula*) or reward type (*Lysimachia*), restricting the number of possible flower visitor species. The main flower visitors of *Phyteuma* and *Scrophularia* are generalists (bumblebees, long-tongued syrphids and solitary wasps) that visit many different plant species, whereas the main flower visitors of *Campanula* and *Lysimachia* are oligolectic solitary bees, thus obligate specialists. Within each pair of plant species, one is more common than the other (table 6.1).

We used potted plants to manipulate patch sizes, and to prevent local effects on seed set, such as soil nutrient availability and humidity. Also the chemical composition of nectar can be affected by local soil nutrient concentrations (Gardener & Gillman 2001). Plants were individually grown in pots from seed or stock, and were given equal watering and fertilisation within species. The plants were kept under insect gauze (without touching the plants) to prevent insect visitation, but allowing watering and sun light.

Environments and sites

All the experimental sites were road verges, except two sites for *Succisa*, which were meadows inside reserves. Sites were selected to introduce patches of the potted plants; the selection was based on earlier observations in the research area (chapters 2-4). For *Anthriscus*, *Succisa*, *Phyteuma* and *Scrophularia* the environments differed in land use: (1) agricultural land use, i.e. monocultures of fertilised and sprayed arable land or grassland without flowering plants interesting for insects, and (2) reserves with semi-natural hay-meadows, with several different flowering, entomophilous plant species. Sites adjacent to intensive agricultural fields were named "agriculture", and sites near reserves were named "reserve". For *Anthriscus* and *Phyteuma*, one agriculture and one reserve site were used, because the number of potted plants that produced flowers was too low for four sites. There were two low biodiversity and two high biodiversity sites for *Scrophularia*.

Apart from land use, the population size was a site feature for the *Succisa*: sites with less than 500 *Succisa* flower heads were considered "small", sites with between 500 and 2500 flower heads "large". There was one "agriculture-large" site, one "reserve-small" site and two "reserve-large" sites. The latter were meadows inside a reserve instead of road verge.

For *Lysimachia* and *Campanula*, sites differed in absence and presence of the target plant species in the vegetation. Earlier experience showed that when the plant species were present, frequently also their oligolectic solitary bees (table 1) would be present (chapters 2 and 3). We reasoned that when the target plant species were absent, the oligolectic bees would also be absent. Sites are named "*Campanula*/ *Lysimachia* absent", or "*Campanula*/ *Lysimachia* present". For *Lysimachia*, the absent site was at our research institute outside the research area: *Lysimachia* was very abundant in the research area, but we wanted to be sure that the oligolectic bee was not present. For *Campanula* there were two *Campanula* absent and two present sites, for *Lysimachia* one *Lysimachia* absent and two present sites.

Table. 6.1. The six plant species used in the experiments: Taxonomy, national and regional red list status and trend, flower biology and flower features, and most important flower visitors. Taxonomy follows (van der Meijden et al. 1996;Tamis et al. 2004). Sources in literature are given in the footnotes. The dominant visitor groups are also estimated by using data from chapters 1 and 2.

Feature	*Anthriscus sylvestris*[4]	*Succisa pratensis*[5]	*Phyteuma spicatum* subs. *nigrum*[6]	*Scrophularia nodosa*[7]	*Campanula rotundifolia*[8]	*Lysimachia vulgaris*[9]
Family	Apiaceae	Dipsacaceae	Campanulaceae	Scrophulariaceae	Campanulaceae	Primulaceae
National status & tendency[1]	Very common Increase	Fairly common Decrease	Very rare Endangered	Common Stable	Common Stable	Very common Stable
Regional status & tendency[2]	Very common Strong increase	Fairly common Strong decrease	Very rare Strong decrease	Fairly common Stable	Common Decrease	Very common Stable
Flowering	Protandrous	Protandrous	Protandrous	Protogynous	Protandrous	Not separated
Self-compatibility	Yes	Yes	No	Yes	Yes	Yes
Selfing	Rarely	No	No	Occasionally	Occasionally	Occasionally
Clonality	Yes, shoots	Yes	Yes	Yes	Yes	Yes, extreme
Flower type, orientation	Umbel, upwards	Head, upwards	Brush, sideways	Gullet, sideways	Bell, downwards	Dish, upwards
Flower colour	White	Blue	Dark violet	Brown	Blue	Yellow
Nectar accessibility	Open	Shallow tubes	Hidden	Hidden	Hidden	No nectar, but oil
Natural flowering period[3]	April- June	July- autumn	May- June	June-September	June- autumn	late June- August
Main visitors	Diptera (other than syrphid flies)	Syrphids (mainly large Eristalinae)	Bumblebees, Rhingia campestris (syrphid)	Bumblebees, Vespidae	Melitta haemorrhoidalis (oligolectic solitary) bee	Macropis europaea (oligolectic solitary bee)
Other visitors	Beetles, syrphids, diverse wasps, ants	Bumblebees, butterflies			Small diptera	Small syrphids

[1] Tamis *et al*. 2004 [2] Stichting Werkgroep Florakartering Drenthe 1999 [3] van der Meijden *et al*. 1996 [4] van Mierlo & van Groenendael 1991;Spalik & Woodell 1994
[5] Adams 1955;Kwak 1993;Vergeer *et al*. 2003a;Hunneman *et al*. 2004 [6] Kwak 1993;Kwak *et al*. 1998;Kwak & Vervoort 2000;Wheeler & Hutchings 2002
[7] Fægri & van der Pijl 1979;de Vos 1983;Koniuszek *et al*. 1986 [8] Fægri & van der Pijl 1979;Nyman 1992;Westrich 1996;Peeters *et al*. 1999;Skov 2000
[9] Vogel 1976;Vogel 1986;Peeters *et al*. 1999

Field experiments

When the plants were flowering, patches with approximately equal flower numbers were introduced into the field sites. The potted plants were flowering at the same time as natural plants in the field. Early in the morning prior to introduction into the field, flower units were individually labelled with numbered tape (*Anthriscus* and *Succisa*) or different paint colour combinations on the flowers or flower bottoms (*Phyteuma*, *Scrophularia*, *Campanula* and *Lysimachia*). The marked flower units were in the female stage of flowering for *Anthriscus* (part of umbellules within umbels only), *Succisa*, *Phyteuma*, *Scrophularia* and *Campanula*. On *Lysimachia* plants, where male and female stages are not separated, flowers that started to open were marked at random.

The patches were installed before insect activity started (usually 11:00, but also earlier, depending on weather conditions and seasonal day length). The artificial patches were placed at least 1 m away from natural patches, in order to maintain the desired patch size. The plants were removed at the end of the day (between 16:30 and 17:30) and were stored again under the insect gauze. The patches were in the field for only one day (except *Lysimachia*), as we wanted to keep patch sizes constant, and the female stage usually lasted for only one day. Keeping the plants in the field for only a short time also prevents theft and vandalism by people.

Anthriscus patches consisted of 14 umbels, all partially in the female stage. At each site, two patches of 14 umbels were introduced on 7 and 8 April 2003.

Succisa patches consisted of 30 flower heads, of which 7 marked heads in the female stage and 23 male flower heads. At each site, one artificial patch was introduced. Instead of a second patch, seven flower heads in the female stage were placed in plastic tubes filled with water attached to a stick, around two meters away from each artificial patch. The female heads were collected in the morning prior to the experiment and were virgin at the start of the experiment. Also the female flowers on the plants in the patches were virgin at the start of the experiment. Virginity was checked with a hand magnifier. After the experiment the female flower heads were collected from the tubes. For each of the latter heads, on 15 stigmas the number of deposited *Succisa* pollen grains was counted using a 15x hand magnifier. Artificial patches and flowers on tubes were introduced at the low biodiversity sites on 18 and 25 September 2003, and at the high biodiversity sites on 18 and 26 September 2003.

Patches of *Phyteuma* consisted of 14 flowering stems. On each stem, three flowers in the female stage were marked. At each site, two patches were introduced. The patches were introduced at both sites on 27 and 29 May 2003.

Scrophularia patches consisted of 100 to 120 flowers, of which between 15 and 20 female flowers in the female stage were individually marked. At each site, two patches were introduced: One pair of low and high biodiversity sites on 17 June 2003, the other pair on 25 June 2003.

Campanula was introduced in 2002 and 2003 for year comparison and it was the only species that flowered abundantly enough to be used in 2002. At each site, two patches consisting of between 25 and 35 flowers were installed. In each patch, between 7 and 10 flowers in the female stage were individually marked. The patches were introduced at sites *Campanula* absent 1 and present 1 on 30 and 31 July 2002, and on 21 and 31 July 2003, and at the sites *Campanula* absent 2 and present 2 on 9 and 12 August 2002, and on 23 July and 5 August 2003.

The male and female stages of *Lysimachia* flowers are mostly not separated (Vogel 1976). We wanted to be sure that pollination could occur; therefore *Lysimachia* patches were kept in the field for three continuous days. At each site, two patches of 70- 85 flowers were introduced on 12 August 2003 before 11:00 h, and removed on 15 August 2003 after 15:00 h. Two to three flowers that started to open were marked in each flowering stem, 16- 22 flowers in total in each patch.

For all plant species, at each site and date the number of entomophilous flowering plant species (herbs, no wind-pollinated plant species like grasses) and flower visiting insect species were counted in transects of 100 m. The introduced patches were in the middle of a transect. The number of insect species visiting inflorescences were counted at the middle of the day by walking slowly along a transect once. Insects were identified in the field to species or morphotype, or samples were taken to be identified later.

The visitation rate (number of visits per unit time) was measured per patch per plant species. During observation periods of seven minutes (*Anthriscus*) or ten minutes (the other plant species), all flower visits to the potted target species were scored per insect species. The number of observation periods per patch per site was between two and six per day. This depended on the time needed to drive between sites to do the measurements.

From the transect observations, species richness of flowering plants and of flower-visiting insects per site per day was calculated for each target plant species. The number of visitor species on the target plants per patch per day was calculated from the visitation rate observations.

Self-pollination

Four of the six target species are known to be self-compatible and capable of autogamy (table 6.1): *Anthriscus* (van Mierlo & van Groenendael 1991), *Scrophularia* (Koniuszek *et al*. 1986), *Campanula* (Nyman 1992) and *Lysimachia* (Vogel 1976, 1986). For these four plant species the importance of insect pollination and the ability to self selfing were measured: random umbels (*Anthriscus*) or individual flowers (*Scrophularia* and *Campanula*) in the female stage were marked on the potted plants. On *Lysimachia*, where male and female stages are not separated, random flowers that started to open were marked. The plants with marked flowers were kept under insect gauze during flowering to prevent visitation. The produced seeds were collected and counted, and germinability was tested (see below). *Succisa pratensis* is self-compatible, but does not produce viable seeds without insect visitation (Adams 1955; Kwak 1993).

Seed set and germinability

Ripe seeds of marked flowers were harvested and counted. Seed viability was estimated by germinability tests: seeds were put into Petri dishes with moist filter paper and kept in climate chambers at a regime of 12 h light at 25^o C and 12 h dark at 15^o C. Prior to this, a stratification treatment at 5° C was given to seeds of *Anthriscus* (six weeks) and *Lysimachia* (four weeks). No effort was taken to test seed germination of *Phyteuma*, as the seeds are notoriously difficult to germinate under laboratory conditions (Wheeler & Hutchings 2002).

Statistics

We used multiple linear regression to analyse the effects of flowering plant species diversity and insect species diversity on insect visitor diversity. Visitation rate, seed set and germination data were analysed with nested mixed design ANOVAs (analysis of variance). The *Lysimachia*-data were tested with univariate ANOVAs. Visitation rate data were square-root transformed in order to meet the assumption of homogeneity of variances. Post hoc effects were tested with Tukey tests. For *Campanula*, the two years were analysed separately. All statistical tests were performed with the package SPSS for Windows version 12.0.1 (2003).

RESULTS

Species diversity and the number of visitor species

Plant and insect species richness tended to be lower at the agriculture sites compared to the reserve sites for *Anthriscus* and *Scrophularia* (fig. 6.1). The *Succisa* sites had a varying number of insect species (9-33), and plant species richness was always lowest at the reserve sites with large *Succisa* populations (reserve-large 1 and 2: 2 plant species, the other sites between 4 and 9). The mean of plant and insect diversity were approximately the same at the *Phyteuma* sites (7 and 20, respectively). The *Campanula* and *Lysimachia* sites had some variation in species richness of plants and insects, but this was not connected to the absence or presence of the target species.

The number of visitor species on the target plants was more than five species for the two generalist species (fig 6.1 A-B) and less than five species for most of the specialist plant species (fig. 6.1 C-F). There was not much variation in the number of visitor species for most of the target plants; it varied most between sites for *Succisa* (between 6 and 13 insect species per patch).

The relation between insect species richness and plant species richness was positive only for *Anthriscus* (table 6.2), and negative for *Succisa*, *Phyteuma* and *Campanula*. The r2-values (table 6.2) were low for most species because there were only few sites and there was little variation. The relation between the number of insect species on all plants at a site and the number of visitor species on the target species was only significant for *Succisa* and *Phyteuma*. The insect species richness between sites for *Phyteuma* varied between 13 and 25 species, the number of insect species visiting *Phyteuma* between 1 and 8. The number of insect visitor species in *Succisa* patches was correlated most with the population size of *Succisa* (table 6.2).

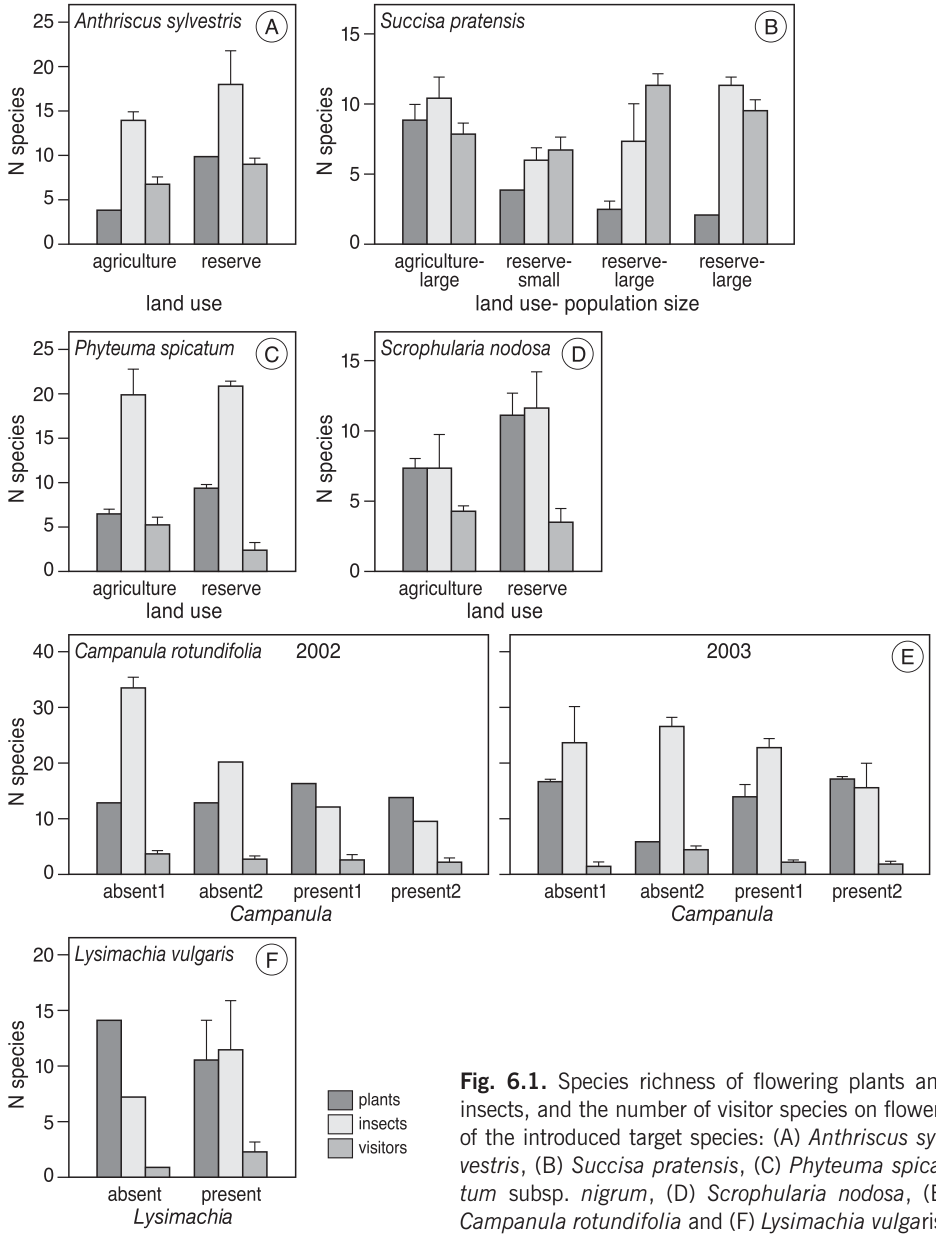


Fig. 6.1. Species richness of flowering plants and insects, and the number of visitor species on flowers of the introduced target species: (A) *Anthriscus sylvestris*, (B) *Succisa pratensis*, (C) *Phyteuma spicatum* subsp. *nigrum*, (D) *Scrophularia nodosa*, (E) *Campanula rotundifolia* and (F) *Lysimachia vulgar*is. Species richness of plants and insects was measured in transects of 100 m, the number of insect species was observed on all plant species at a site. The number of visitor species was measured during observations of visitation rate in introduced patches with potted plants of the target plant species. The patches were introduced into road verges in environments that differed in land use (A, C and D), land use and population size of *Succisa* (B) and absence/ presence of the target species, being a measure of the absence/ presence of oligolectic bees that are the main visitors (E and F). For *Succisa* (B) the environments "reserve-large" were in meadows inside a reserve. See text for further explanations.

Table. 6.2. Multiple regressions for the relation between total species richness of plants and insects, and the number of visitor species (insects) on flowers of the introduced target species. Species richness of plants and insects was measured in transects of 100 m, the number of insect species was observed on all plant species at a site. The number of visitor species was measured during observations of visitation rate in introduced patches with potted plants of the target plant species. For Succisa also the relation between population size and the number of visitor species was analysed with a simple linear regression. There were not enough data points for a regression with *Lysimachia vulgaris*.

				insect species		Target species	
	r^2	F	p	β	p	β	p
Anthriscus sylvestris	0.56	7.40	0.03	1.01	0.01		n.s.[1]
Succisa pratensis	0.33	4.75	0.03	-0.55	0.03	0.52	0.03
Succisa pratensis (flower abundance)	0.64	27.84	0.00	0.82	0.00		
Phyteuma spicatum subsp. *nigrum*	0.72	10.12	0.02	-0.62	0.03	0.65	0.02
Scrophularia nodosa		n.s.[1]					
Campanula rotundifolia	0.24	5.94	0.01	-0.49	0.07		n.s.[1]

[1] not significant

Visitor composition

The six plant species had different compositions of visits (fig. 6.2), and for all target plant species there was some variation in the proportions of visits by different visitors. At all sites, the main visitors for *Anthriscus* (fig. 6.2A) were non-syrphid Diptera (Calyptrata, Empididae and Bibionidae). At the reserve site, umbels received relatively more visits from *Empis* species than at the agriculture site.

The main visitors on *Succisa* flower heads were bumblebees (mainly *Bombus pascuorum*) and Eristalinae (large syrphid flies, i.e. *Eristalis* spec. and *Helophilus* spec.). The proportion of visits by Eristalinae was highest at the site agriculture-large (fig. 6.2B). At the site reserve-small, around 60% of the visits were from bumblebees. At the reserve-large sites, 30 - 50% of the visits were from Eristalinae, and 30% from bumblebees. At the reserve-large site was also the largest proportion of honeybee and butterfly visits.

Phyteuma received most of the visits from the long-tongued syrphid *Rhingia campestris* (fig. 6.2c). The largest proportion of bumblebee visits was at the reserve site. At the agriculture site, around a third of the visits was from the polylectic solitary bee *Andrena haemorrhoa*.

The main visitors on *Scrophularia* (fig. 6.2D) were wasps (*Dolichovespula sylvestris*) and bumblebees (*Bombus pascuorum* and *B. pratorum*). The number and proportion of wasp visits was higher at the agriculture site.

There was considerable variation in the composition of visits between the sites and the two years for *Campanula*. Contrary to what was expected, the oligolectic bee that was thought to be excluded in the site absent 2, *Melitta haemorrhoidalis*, visited flowers of *Campanula* (only one day in 2002). An inspection of the vicinity revealed that *Campanula rotundifolia* was flowering ca. 400 m away from the introduced patch. In 2003, no *Campanula* flowers were present, and the bee was not observed. In 2002 (fig. 6.2E),

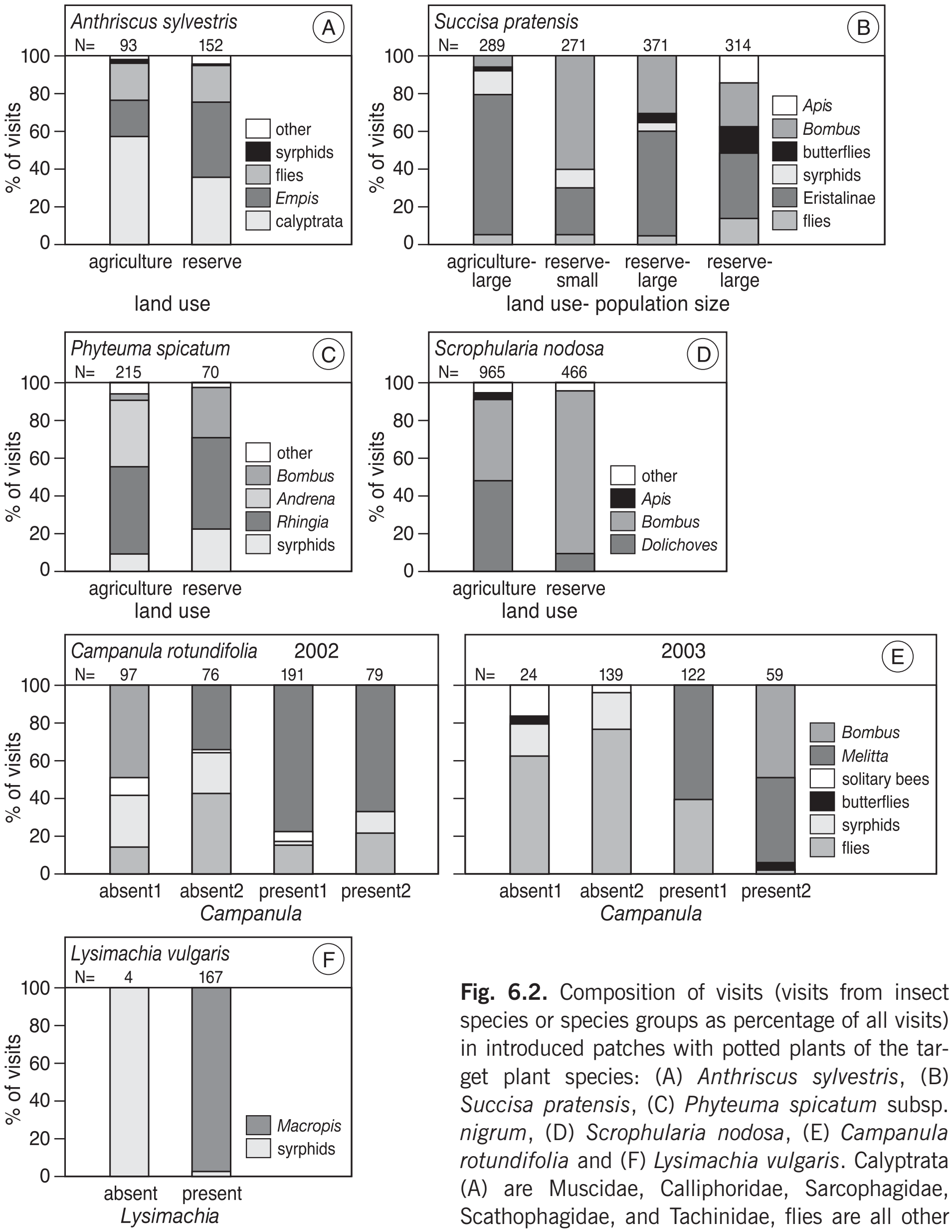


Fig. 6.2. Composition of visits (visits from insect species or species groups as percentage of all visits) in introduced patches with potted plants of the target plant species: (A) *Anthriscus sylvestris*, (B) *Succisa pratensis*, (C) *Phyteuma spicatum* subsp. *nigrum*, (D) *Scrophularia nodosa*, (E) *Campanula rotundifolia* and (F) *Lysimachia vulgaris*. Calyptrata (A) are Muscidae, Calliphoridae, Sarcophagidae, Scathophagidae, and Tachinidae, flies are all other diptera than Calyptrata, *Empis*-species and syrphids. *Apis* (B, D) is the honeybee *Apis mellifera. Bombus* (B- E) are bumblebees. Eristalinae (B) are larger syrphid species. *Andrena* (C) is the polylectic solitary bee *Andrena haemorrhoa. Rhingia* is the long-tongued syrphid *Rhingia campestris. Dolichovespula* (D) are wasps of the Vespidae. *Melitta* (E) is the oligolectic solitary bee *Melitta heamorrhoidalis*, specialised on *Campanula*, "solitary bees" are other polylectic and oligolectic species. *Macropis* (F) is the oligolectic solitary bee *Macropis europaea* specialised on *Lysimachia*. For all species, N is the number of visits for all oberservation periods combined. The explanations of the x-axes are in fig. 6.1, further explanations can be found in the text.

more than 70% of the visits at the sites with *Campanula* (present 1 and 2) and 30% in site absent 2 were from *M. haemorrhoidalis*. At the site absent 1, *Bombus pascuorum* and polylectic solitary bees (*Megachile* species) were bringing 60% of the visits. The rest of the visits was from *Rhingia campestris* and small Diptera. In 2003, more than 60% of the visits at the *Campanula* present sites were from *M. haemorrhoidalis*. The majority at the *Campanula* absent sites was from *Rhingia campestris* and small Diptera, and between 5 and 15 % of the visits was from very small (~4 mm) solitary bees.

For *Lysimachia*, more than 90% of the visits at *Lysimachia* present were from the oil collecting oligolectic solitary bee *Macropis europaea* (fig. 6.2F). The rest of the visits and all visits at *Lysimachia* absent were from small syrphids (*Sphaerophoria scripta* and *Syrphus* spec.).

Visitation rate

Although the visitation rate varied between sites for several target plant species (fig. 6.3), this was only significant for *Scrophularia* and *Campanula*. Visitation rate did not differ for *Anthriscus* ($F_{1,2}$=1.5, $p > 0.3$), *Succisa* ($F_{3,4.01}$=0.06, $p > 0.9$), and *Phyteuma* ($F_{1,2}$=5.5, $p > 0.1$). It also did not vary for *Lysimachia* (F_2=3.3, $p > 0.1$), in spite of the fact that the mean visitation rate at *Lysimachia* present was 10-25 times higher than at *Lysimachia* absent (fig. 6.3F).

The agriculture site of *Scrophularia* had a two times higher visitation rate (fig. 6.3d) than the reserve site ($F_{1,4.03}$=10.3, $p < 0.05$). The difference in visits is mainly caused by wasps: flowers received more visits from wasps at the agriculture sites, than at the reserve sites, whereas the number of bumblebee visits was approximately equal.

For *Campanula*, the total visitation rate and the visitation rate for bees separately (all solitary bees and bumblebees) were analysed (fig. 6.3E). The total visitation rate did not differ between the sites in 2002, although *Campanula* present 1 tended to be highest ($F_{3,4.02}$=1.2, $p > 0.4$, fig. 6.3E). We also analysed visitation rate for bees separately, but this did not differ between sites either ($F_{3,4.05}$=2.1, $p > 0.2$). The visitation rate in 2003 differed only between sites absent 1 and absent 2 ($F_{3,4.4}$=11.1, $p < 0.05$). The visitation rate for bees separately was lowest at the site *Campanula* absent 2, and highest at the sites present 1 and 2 ($F_{3,4.5}$=15.8, $p < 0.01$).

Selfing, seed set and germination

Insect visitation and pollination lead to significantly higher seed set for all four species in which selfing capability was analysed (*Anthriscus*, *Scrophularia*, *Campanula* and *Lysimachia*). The number of seeds produced and germinated from flowers that were excluded from insect visitation was much lower than from the flowers with insect visitation (fig. 6.4). However, in all four plant species some flowers excluded from insect visitation produced some viable seeds: of the *Anthriscus* umbels, 80% produced seeds, but fewer seeds per umbel than the visited umbels (around 14 compared to 50 seeds), and only 17% of the produced seeds germinated. Around 30% of the excluded *Scrophularia* flowers produced seeds, much less than the visited flowers (30 compared to 80). In 2002, around 75% of the *Campanula* flowers excluded from insect visitation produced seeds,

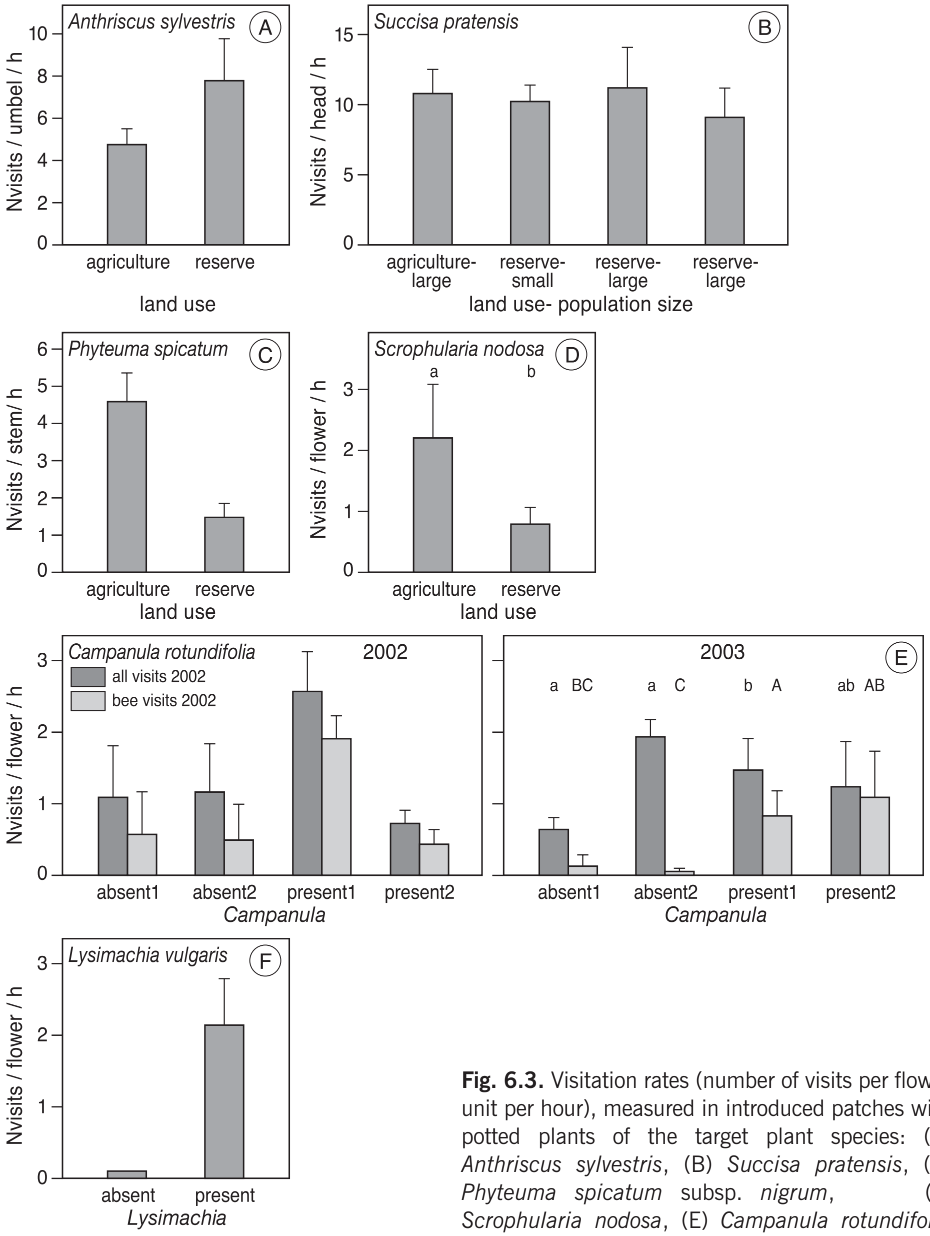


Fig. 6.3. Visitation rates (number of visits per flower unit per hour), measured in introduced patches with potted plants of the target plant species: (A) *Anthriscus sylvestris*, (B) *Succisa pratensis*, (C) *Phyteuma spicatum* subsp. *nigrum*, (d) *Scrophularia nodosa*, (E) *Campanula rotundifolia* and (F) *Lysimachia vulgaris*. Means were calculated over observation periods of 7 minutes (*Anthriscus*, A) or 10 minutes (the other plant species, B-F). For *Campanula* (E) visitation rate was also calculated for bees separately (i.e. *Melitta haemorrhoidalis*, other solitary bees and bumblebees combined). Different letters above bars indicate significant differences, in fig. E (right) small letters are for total visitation rate, capitals for bee visitation rate. The explanations of the x-axes are in fig. 6.1, further explanations can be found in the text.

but much less than the flowers visited by *Macropis europaea* (9 vs. 40). In 2003, no excluded *Campanula* flower produced any seeds. Around 12% of the excluded *Lysimachia* flowers produced seeds, slightly less than the flowers with visitation by *Macropis europaea* (8 vs. 10).

The number of seeds of the excluded umbels was significantly lower than of the visited flowers for *Anthriscus* (fig. 6.4), but did not differ between the two treatments of the introduced patches ($F_{2,76}$=27.4, $p < 0.001$). The same was true for germination ($F_{2,76}$=21.3, $p < 0.001$). The germination of the produced seeds was very low: between 6% and 13%.

Succisa flowers always produce seeds, but unfertilised seeds are not viable, therefore the number of developed (viable looking) seeds was counted as well. The proportion of developed seeds was around 30% for all treatments (fig. 6.4B). The number of developed seeds for *Succisa* did not differ between the sites ($F_{3,51}$=0.3, p. 0.8). The proportion of germinated seeds was very low in all sites, and was less than 1% for the two second days that the experiment was conducted (25 and 26 September). Therefore the number of germinated seeds was tested for the whole period, and separately for the two first days (17 and 18 September). Germination was higher at reserve-large 1 compared to agriculture-large for all days ($F_{3,51}$= 3.1, $p < 0.05$). For the first days separately (data not shown), the number of germinated seeds was lower in agriculture large compared two reserve-large 1 and 2, and lower in reserve-small compared to reserve-large 1 ($F_{3,24}$= 6.7, $p < 0.005$).

Phyteuma did not show any differences in seed set ($F_{1,2}$=0.3, $p > 0.6$, fig. 6.4C).
For *Scrophularia* the number of seeds of the excluded flowers was significantly lower than of the visited flowers (fig. 6.4D), but did not differ between the two treatments of the introduced patches ($F_{2,170}$=50.1, $p < 0.001$). The same was true for the number of germinated seeds ($F_{2,170}$=45.1, $p < 0.001$). Germination varied between 77% and 89% of produced seeds for all treatments.

For *Campanula*, in both years significant differences in seed set and germination were found (fig. 6.4e). In 2002, seed set was higher at *Campanula* present than all other sites and the flowers excluded from insect visitation ($F_{4,137}$=5.8, $p < 0.001$). The number of germinated seeds was higher at *Campanula* present 1 than at absent 1 and 2 and the flowers excluded from insect visitation ($F_{4,137}$=5.4, $p < 0.001$). Germination varied between 54% and 75%. In 2003, seed set was highest in *Campanula* present 1 and 2, and there was no significant difference between absent 1 and 2, and the flowers excluded from insect visitation ($F_{4,112}$=13.7, $p < 0.001$). For the number of germinated seeds the same differences were found ($F_{4,112}$=11.6, $p < 0.001$). Germination varied between 75% and 81%.

Seed set of *Lysimachia* was higher at *Lysimachia* present than at *Lysimachia* absent and the flowers excluded from visitation (fig. 6.4F), but there was no difference between *Lysimachia* absent and flowers excluded from visitation ($F_{2,196}$=33.3, $p < 0.001$), this was the same for the number of germinated seeds ($F_{2,196}$=33.3, $p < 0.001$). The proportion of germinated seeds of seeds produced was between 73% and 96%.

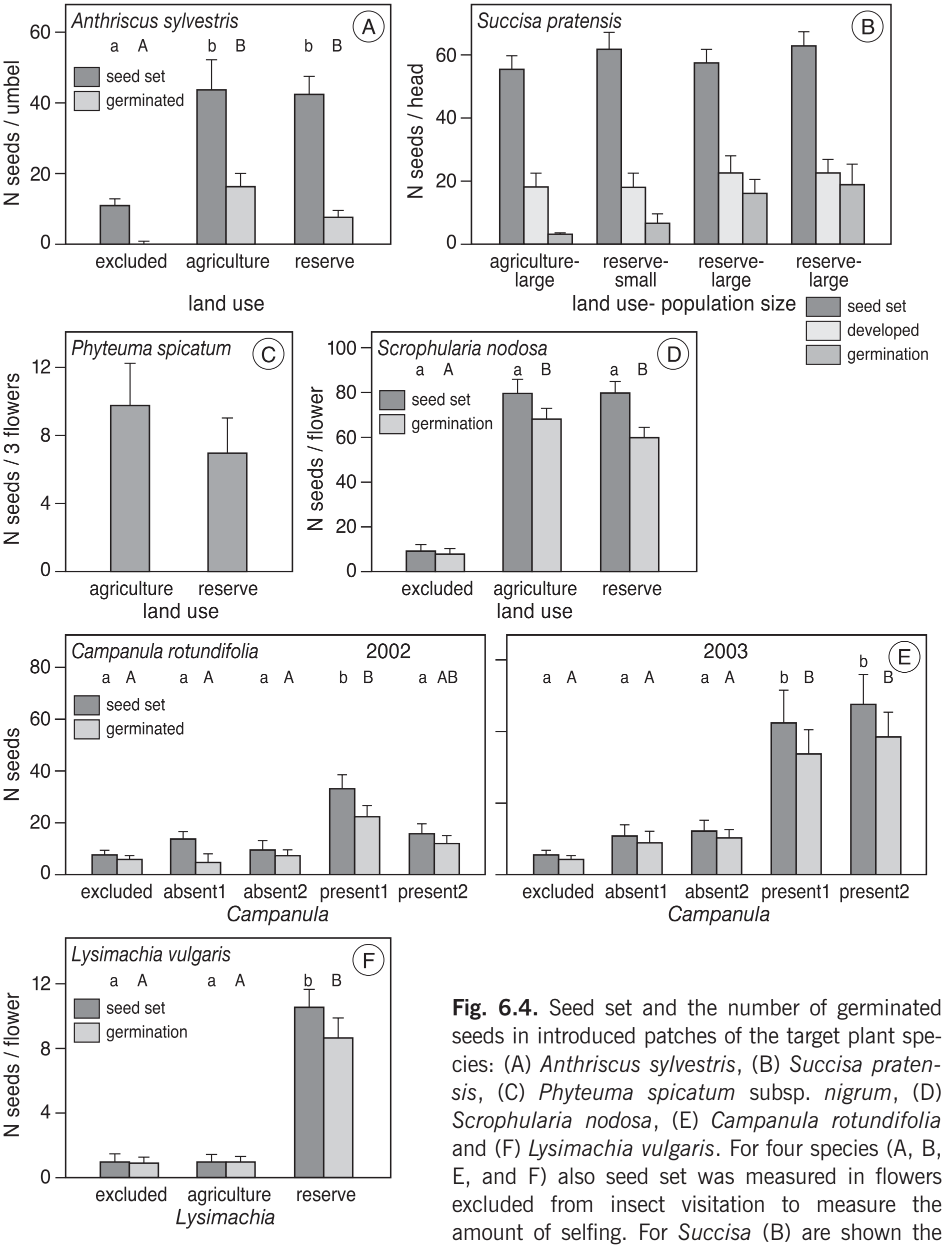


Fig. 6.4. Seed set and the number of germinated seeds in introduced patches of the target plant species: (A) *Anthriscus sylvestris*, (B) *Succisa pratensis*, (C) *Phyteuma spicatum* subsp. *nigrum*, (D) *Scrophularia nodosa*, (E) *Campanula rotundifolia* and (F) *Lysimachia vulgaris*. For four species (A, B, E, and F) also seed set was measured in flowers excluded from insect visitation to measure the amount of selfing. For *Succisa* (B) are shown the total number of seeds produced (seed set), the number of seeds counted as viable looking (developed) and the number of germinated seeds (germination). Different letters indicate significant differences, small and capital letters are for seed set and the number of germinated seeds respectively. The explanations of the x-axes are in fig. 6.1, further explanations can be found in the text.

Pollen deposition (*Succisa*)

The number of deposited pollen did not differ significantly (fig. 6.5), but agriculture-large and reserve-small tended to have a lower number of deposited pollen grains compared to the two reserve-large sites ($F_{3,4}=3.6$, $p> 0.1$): around 10 and 5 pollen grains per stigma per head compared to around 13 and 14 pollen grains.

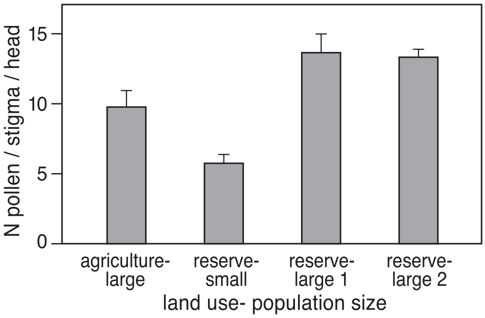


Fig. 6.5. Pollen deposition of *Succisa pratensis* pollen grains on introduced flower heads of *Succisa pratensis*. Seven flower heads in the female stage were placed in plastic tubes filled with water attached to a stick at each site. The female heads were collected in the morning prior to the experiment and were virgin at the start of the experiment. The explanations of the x-axes are in fig. 6.1, further explanations can be found in the text.

Discussion

Insect pollination vs. self-pollination

The "escape" from pollinator limitation through selfing (Kearns *et al*. 1998; Kwak *et al*. 1998; Spira 2001; Donaldson *et al*. 2002) may be less secure for plants than previously thought: Seed set of the four plant species with potential self-pollination capacity (*Anthriscus sylvestris*, *Scrophularia nodosa*, *Campanula rotundifolia* and *Lysimachia vulgaris*) was considerably reduced without insect visitation. This implies that animal or insect pollination may be crucial for reproduction through seeds even for self-compatible and facultative selfing plant species, and that studying insect pollination is worthwhile in those species. *Succisa pratensis* is self-compatible, but needs insect pollination, and *Phyteuma spicatum* subsp. *nigrum* is self-incompatible.

Visitors, visitation rates and seed set

The introduction of the target plants into different environments induced differences in the number of visitor species and the visitor compositions in all six target plant species. The variation in the number of visitor species could be related to flowering plant species richness in four target species, and to insect community diversity in two plant species (table 6.3). The number of visitor species varied most in *Succisa*, which was probably mainly

Table. 6.3. Conclusions from the results for the six target plant species in this study. The first two yes/no questions concern the relation between plant and insect community diversity and visitor diversity on the target plants, questions 3-4 are about the effects of the environments (see material and methods), and in question 6 is asked whether the plant species have alternative pollinators.

	Anthriscus sylvestris	*Succisa pratensis*	*Phyteuma spicatum*	*Scrophularia nodosa*	*Campanula rotundifolia*	*Lysimachia vulgaris*
1.Correlation flower diversity and visitor diversity?	yes +[1]	(yes -)[2]	yes -	no	(yes -)[3]	x[4]
2. Correlation insect diversity and visitor diversity?	no	yes +	yes +	no	no	x[4]
3. Composition of visits different?	yes	yes	yes	yes	yes	yes
4. Is visitation rate affected?	no	no	no	yes	yes	(yes)[5]
5. Are seed set or germination affected?	no	yes	no	no	yes	yes
6. Are pollinators irreplaceable?	no	no	no	no	yes	yes

[1] Positive correlations: +, negative correlations: -. [2] Low r^2, population size of Succisa is better correlated. [3] Low r^2. [4] Not analysed. [5] Not significant .

due to the variation in the population size of *Succisa* itself at the sites. For practical reasons there were only a limited number of sites and repeats per plant species, making the regression analyses (table 6.2) rather weak.

For both generalist and specialist plant species the composition of visits varied: *Phyteuma*, *Campanula* and *Lysimachia* were visited by different insect groups at the different sites and environments. The proportions of visits from various visitor taxa varied between the sites for *Anthriscus*, *Succisa* and *Scrophularia*, but for *Anthriscus* only marginally. Variation between years was found for *Campanula*: bumblebees were visiting in 2002 only, and the number of *Melitta haemorrhoidalis* individuals varied. Thus our results confirm the variability of visitor species on plants in space and time (Corbet 1997; Kearns *et al*. 1998).

Differences in visitation rate were found for three specialist plant species (table 6.3), including both target species with specialist visitors (*Scrophularia*, *Campanula* and *Lysimachia*). For *Scrophularia*, the visitation rate was higher in the agriculture sites, where biodiversity in the sites themselves and in the environment were lower than in the reserve sites. For *Campanula*, the difference became more apparent when the visits of bees were analysed separately. The difference was not significant for *Lysimachia*. In the other three species, visitation rate did not differ between the environments because different visitor compositions at the sites led to the a similar result. Thus concerning visitation rate of the latter three plant species, insects may be exchangeable.

The effects of the environments on seed set were partially opposite compared to those on visitation rate: For *Scrophularia*, visitation rate differed, but seed set was not affected and for *Succisa* seed set showed differences, while there were no effects on visitation rate (table 6.3). Although the sites did not differ in visitation rates, this did result in similar pollination quality for *Succisa*: the sites with the lowest seed se*t al*so tended to show the lowest pollen deposition. Only for *Anthriscus*, *Phyteuma* and *Lysimachia* the results for visitation rate and seed set and germination had the same direction. The opposing results for visitation rate and seed set of some of the target species indicate that visitation rate alone may not always be a good measure of pollination effectiveness, but should be combined with seed set and behavioural observations.

Alternative pollinators

Whether pollinators of a plant species can be replaced depends on the availability and pollination quality of alternative pollinators (Kwak *et al*. 1998). *Anthriscus* has a large number of alternatives, as it is visited by many species, similar to other Apiaceae species (Ellis & Ellis-Adam 1993, 1994; chapter 2). The pollinator quality of some abundant visitors (flies, syrphids and beetles) varies only little (Kwak & de Vlas, unpubl.). Therefore it is not surprising that we did not find any effects of the environment on visitation and seed set of *Anthriscus* in our study.

Also *Succisa* has a broad range of visitors, but the quality of those visitors varies: the main visitors are large syrphids, mainly *Eristalinae* (Kwak 1993; Hunneman *et al*. 2004), but also bumblebees (Kwak 1993). In Europe, including the Netherlands, several oligolectic bee species exist that are specialised on Dipsacaceae genera, i.e. *Scabiosa, Knautia* and *Succisa* (Westrich 1990). In the past, *Andrena marginata* used to be a common visitor on *Succisa pratensis* in the south and east of the Netherlands, including the research area (Peeters *et al*. 1999). However, it is extinct in the Netherlands since the 1960s, and declining in other European countries (Peeters *et al*. 1999; Peeters & Reemer 2003). It is likely that *Succisa* has already lost one of its main pollinators in the Netherlands, but information about its pollination quality is not available. However, the pollination quality of oligolectic bees is known for a related plant species, indicating the probability of the importance of solitary bees as pollinators: The bee Dasypoda argentata was by far the most efficient pollinator for Scabiosa columbaria in France (Velterop 2000).

Most visits to *Phyteuma* were from the syrphid *Rhingia campestris*, but the main pollinators are bumblebees with short to intermediate probosces (Kwak 1993, 1994b; Kwak & Vervoort 2000). Our results showed that solitary bees may be alternative pollinators in the absence of bumblebees: neither visitation rate nor seed set differed between the environments. is unlikely to be an alternative, as its pollination quality for *Phyteuma* is very poor compared to bumblebees (Kwak 1993).

Scrophularia has typical "wasp" flowers (Fægri & van der Pijl 1979), and indeed, de Vos (1983) demonstrated that Vespidae (*Dolichovespula* species) are efficient pollinators, together with bumblebees. In our study, a difference in visitation rate did not result in higher seed set. The visitation rate was probably high enough at both sites to be sufficient for seed set, and differences in visitation rate did not matter. Still, we may conclude at this point that *Scrophularia* has a number of alternative pollinators, i.e. several bumblebee and wasp species and the honeybee.

The oligolectic bee Melitta *haemorrhoidalis* clearly was the crucial pollinator for *Campanula* in our study. Visitation by flies and (bumble)bees resulted in similar visitation rates, but low seed set. The role of small flies is negligible: they hardly touched stigmas, and the visitation speed of *Melitta* is 10 to 100 times higher than that of the flies (F. Hoffmann, unpubl.). However, several studies showed that in different regions the most important pollinators for *Campanula* differ: oligo- and polylectic solitary bees, bumblebees or large flies lead to successful pollination (Bingham & Orthner 1998; Bingham & Ranker 2000; Blionis & Vokou 2001). In Europe, there are several bee species from different genera and with different body sizes that are oligolectic foragers on *Campanula* species (Westrich 1990; Peeters *et al*. 1999) In Denmark twenty bee species were observed on *Campanula*, of which only three were oligolectic (Skov 2000). So, although *Campanula* may have many potential pollinators, many of them are unreliable due to local rarity and regional differences in occurrence, or their generalist foraging behaviour.

Lysimachia is a typical oil-producing plant and closely connected to oil-collecting flower visitors. In Europe there are only two oil-collecting bee species, *Macropis europaea* and *M. fulvipes* (Vogel 1976, 1986; Fægri & van der Pijl 1979; Celary 2004). Syrphids and other flies are frequently observed on *Lysimachia* flowers, but as pollinators they are no alternative for the bees. *Macropis europaea* has much higher visitation speed than syrphids (M.M. Kwak, unpubl.), and the results from our observations are evident. In the Netherlands, *M. fulvipes* is very rare and restricted to the south. It differs from *M. europaea* in phenology (earlier), and is observed on other *Lysimachia* species in Central and Eastern Europe (Vogel 1986; Pekkarinen *et al*. 2003; Celary 2004). Macropis bees visit *Lysimachia* for pollen and oil, but need to forage on other plant species for nectar (Vogel 1986; Celary 2004). We observed it on at least four plant species: *Lythrum salicaria, Lycopus europaea, Mentha aquatica* and *Cirsium arvense*. Thus indirectly, *Lysimachia* also depends on the presence of nectar plants in the vicinity, and thus on plant species diversity.

Ecological specialisation

Our results confirm the hypothesis that specialist plant species with few specialist pollinators are the most vulnerable to pollinator loss (Rathcke & Jules 1993; Bronstein 1995; Kwak *et al*. 1998; Johnson & Steiner 2000): for *Campanula* and *Lysimachia* the absence of their oligolectic bees had a negative effect on visitation rate, seed set and germination.

Specialisation in this paper is regarded as ecological specialisation of a plant species, referring to having few pollinators relative to other plant species. Ecological specialisation is a state, contrary to evolutionary specialisation, which is a process with a direction, i.e. from many to less pollinating taxa (Armbuster *et al*. 2000). The dichotomy of specialisation vs. generalisation is actually a continuum (Waser *et al*. 1996; Armbuster *et al*. 2000; Johnson & Steiner 2000), this is also the case in this study: all six target species differ in the degree of specialisation for pollination. In order of increasing specialisation, i.e. the number of potential pollinators, the target species are: *Anthriscus* (many different visitor species with similar pollinator quality), *Succisa* (several pollinator species with differing pollinator quality), *Phyteuma* (a limited set of pollinators, but more than *Scrophularia*), *Scrophularia* (pollinators only bumblebees and wasps), *Campanula* (several oligolectic bee species, and sometimes other groups) and *Lysimachia* (exclusively bees of the genus Macropis). The two species at the extremes of the degree of specialisation had opposite results: no effect was found for *Anthriscus*, the generalist with only unspecialised flower visitors, and negative effects for the specialist plant with a single specialised flower visitor. For the other plant species the results were less straight-forward.

A similar conclusion was drawn in a South-African field study, where seed set of seven very different plant species was studied in relation to habitat fragmentation and presence of pollinators (Donaldson *et al*. 2002). The responses were plant species-specific and could not be directly related to pollination system. Only specialised plants with few pollinator species seemed to be the most vulnerable to fragmentation and absence of pollinators. Vázquez & Simberloff (2002) confirm the difficulty to generalise about the relation between the degree of specialisation and the response to disturbance.

The vulnerability of specialised plants should be regarded in context with the degree of specialisation of the pollinators (Ashworth *et al*. 2004), which is what we did in our study: We subdivided the six target plant species into three groups, i.e. generalised plants with generalised visitors, specialised plants with generalised visitors, and specialised plants with specialised visitors. *Scrophularia* and *Phyteuma* both have generalist pollinators, but *Campanula* is dominated by specialised pollinators and therefore fits into the same category as *Lysimachia*. *Succisa* may be in the position of a generalist plant that has lost a specialist pollinator. This may place it in the same position as specialist plant species with generalist pollinators (Ashworth *et al*. 2004). Such simplified systems that have lost part of their pollinator species may be very vulnerable to even further change, as they have already lost some of their pollinator species (Waser *et al*. 1996).

Commonness, conservation and management

The commonness differs strongly between the six target species (table 6.1). *Anthriscus* belongs to one of the 30 most common plant species in the Netherlands (Tamis *et al*. 2004) and is actually a problem for agriculture (van Mierlo & van Groenendael 1991) and for nature conservation in other countries (Hansson & Persson 1994; Dover 1996). It can expand easily through clonal propagation and profits from increased nitrogen deposition (van Mierlo & van Groenendael 1991). The same increase in nitrogen deposition, combined with habitat destruction, causes that the other generalist, *Succisa*, is rapidly declining throughout the Netherlands (Soons 2003; Vergeer *et al*. 2003a). Also the status of the other target plant species differs in the research area: *Phyteuma* is extremely rare and declining in the research area, *Scrophularia* is common, *Lysimachia* very common, and *Campanula* is common, but declining steeply (Stichting Werkgroep Florakartering Drenthe 1999; Tamis *et al*. 2004).

The pollination quality of a plant can change as a consequence of a change in habitat conditions and population size and structure (Kwak *et al*. 1998; Velterop 2000), thereby enhancing a decrease of populations that can result in the population's death-blow. Pollinators will often disappear before the plant, as extinction may be delayed by clonality and long life spans, or blurred by annual variation (Corbet 1997; Johnson & Steiner 2000; Spira 2001).

Current commonness of a plant species is no guarantee for survival in small habitat fragments, as genetic effects may already be on their way (van Rossum *et al*. 2002). This was shown for *Succisa* (Soons 2002; Vergeer *et al*. 2003a & b; Hooftman *et al*. 2004) and *Phyteuma* (Kwak 1994b; Kwak *et al*. 1998). For *Campanula* it may become apparent soon. For *Lysimachia* this is not very likely in the Netherlands, as bee and plant are very abundant. But habitat change is a potential threat: the dependence on specialist pollinators makes that plants also depend on the habitat of their pollinators, as nesting sites for bees and wasps, or the food sources of larvae of other insects, are often in other microhabitats than the plant. For the insects this is called the partial habitat concept (Westrich 1996). For example, some of the insects in our study need to have the following nesting or larvae requirements: *Melitta haemorrhoidalis*, the bee for *Campanula*, requires dry places with open sand for their nests (Westrich 1990), while *Macropis europaea* builds nest in the soil hidden under mosses or tight grass tussocks (Vogel 1976; Celary 2004). Many bumblebee species and *Dolichovespula* wasps need abandoned vole and mole holes for their nests (Heinrich 1979; Goulson *et al*. 2001; Bäckman & Tiainen 2002) and may even depend on population cycles of voles (Vepsäläinen & Savolainen 2000). The larvae of syrphid flies can have requirements as different as various aquatic rotting materials or aphids in pine trees (van der Goot 1981; Verlinden 1991; Stubbs & Falk 1996).

Phenological synchrony with the flowering period of the host plant is a condition for survival of oligolectic bees (Bronstein 1995; Skov 2000). Mowing changes the flowering phenology of plants when they reflower after mowing (chapter 3, box 9.1) or may even shift their flowering phenology on a small evolutionary scale when mowing is consistent for many years (Lack in Proctor *et al*. 1996; ter Borg 1972). Also grazing and climate change will do this, which can cause bee species to disappear due to phenological mismatch (Bronstein 1995). Early and frequent mowing is assumed to be one of the reasons for the extinction of the oligolectic bee *Andrena marginata* (specialised on *Succisa*) in the Netherlands (Peeters *et al*. 1999).

Experimental constraints

In this study we aimed at giving the different target plant species the same treatments in the field. However, this appeared to be difficult to achieve: complications involved the differences in flower biology, differences between sites, exact timing of flowering of the potted plants with plants in the field, and logistical problems concerning transportation. Our study has shown that even within larger studies, plant species will need an individual approach to elucidate the processes involved. In spite of this, we were able to make some generalisations across plant species with different pollination modes. More comparative field studies are needed on a larger scale and during several years. Experiments with artificially modified habitats are another possibility. Furthermore, more information is needed about the efficiency of pollinators. The individual behaviour of pollinators, and therefore pollinator quality, may also change due to habitat alteration. This may lead to a decreased pollination service for some plant species without the actual disappearance of the pollinators (Kwak *et al*. 1998). To study those processes is a daunting, but not impossible task, that requires extensive logistics and will be labour-intensive.

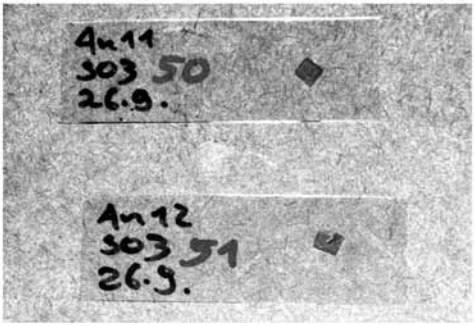
Conclusions

Insect pollination is necessary for seed production, enabling long-term maintenance and seed dispersal of plant species, including facultative selfing species. For very common and extremely generalist plant species, pollinators can be replaced, as there will always be some visitors present acting as pollinators. For plant species with few specialist pollinator species there are hardly any alternatives. For the remaining generalist and specialist plant species with generalist pollinators it is currently difficult to predict the vulnerability to pollinator loss. Studies of individual plant species are still needed in order to make predictions about pollination and the survival chances of plants. For generalisations across species, large-scale studies over several years are needed. For conservation of plants, particularly in fragmented habitats, the habitat requirements of pollinators and the phenology of both flowering plants and their pollinators should be taken into consideration.

ACKNOWLEDGEMENTS

We would like to thank the Dutch State Forestry (SBB) for giving permission to work in their reserve, Jacob Hogendorf for taking care of the plants in the nursery, and Harmen Kraai for help in the field. This study was funded by the Stimulation Program Biodiversity of the Netherlands Organisation for Scientific Research- Earth and Life Sciences (NWO-ALW).

50 Objektträger



An11
303 50
26.9.
An12
303 51
26.9.

7

Pollination quantity and quality in relation to plant population size, flower diversity and flower constancy of syrphids in the devil's bit scabious *Succisa pratensis* (Dipsacaceae)

Manja M. Kwak, Frank Hoffmann and Henk Hunneman

SUMMARY

The effect of population size and the biotic environment (i.e. a low or a high diversity of other flowering plants) on visitor guild, visitation rate and pollen deposition are analysed in nine Dutch *Succisa pratensis* populations in 2002 and 2003. Insect species frequencies and visitation rate, flower constancy as visitation sequences and as body pollen-load composition, pollen composition of stigmatic loads and the number of *S. pratensis* pollen per stigma were measured. Between 5 and 20 insect species per population and 34 in total were visitors of *S. pratensis*. Twelve insect species contributed to >75% of the visits to *S. pratensis*, most of which were syrphids and bumblebees. In 2002, 1.4% of the total number of visits and in 2003, 29.5% were made by bumblebees. The visitation rate varied between 5 and 18 visits per *S. pratensis* head per hour. Large populations of *S. pratensis* were visited by more insect species, but visitation rates did not differ between *S. pratensis* populations. Syrphid species differed in their flower constancy, with *Helophilus pendulus* being a less flower-constant species compared to *H. trivittatus* and *Eristalis horticola*. In small populations the average syrphid load contained between 23 and 18.5% *S. pratensis* pollen, in large populations between 46.5 and 54%. The body loads contained also pollen of plant species from at least 300 m away from *S. pratensis* populations. The percentages of conspecific pollen on stigmas was less (60%) in smaller than in larger populations (70 - 100%). Stigmas had received between 5 and 17 pollen grains at the end of the day, small populations had received significantly less conspecific pollen than large populations. Potential seed set varied between 65% (small populations) and 94% (large populations) of the flowers per head.

The quality of pollination and not the number of visits in large populations was more favourable for seed set than in small populations, with a strong effect of population size and much less of the diversity of the environment. If heterospecific pollen deposition leads to a lower seed set, then small populations in a diverse environment will have the lowest percentage of viable seeds per head, and large populations in an environment with no other simultaneously flowering species will have the highest percentage of seed set.

Andrena marginata, a specialist bee foraging on *S. pratensis*, is extinct in the Netherlands. If it was a better pollinator than syrphids and bumblebees, then the shift to a more generalist pollinator assemblage of only syrphids and bumblebees had negative consequences for the pollination of *S. pratensis*. There may be a positive feedback of declining plant populations, affecting the abundance and efficiency of pollinators, thereby speeding up the decline of the plant population. The abundance or absence of specialist pollinators can be good indicators of declining plant population sizes.

INTRODUCTION

Both plants and animals are decreasing in number of species and in population size, as a result of human impact. The vulnerability of plants to extinction is somewhat hidden by the fact that plants may survive for many years without recruitment (Oostermeijer *et al*. 1992) and by the fact that a population may persist as seed in the seed bank although adult plants have disappeared (Bekker *et al*. 1999). Insects, acting as herbivore or pollinator, are supposed to be faster indicators of declining plant population size, because they depend on the green parts or the flowers of plants (Kevan 1999). In case of pollinators, there may be even a risk of a positive feedback: a declining plant population size may affect the abundance and efficiency of pollinators, and this change in turn may speed up the decline of the plant population. To further explore this idea, we need detailed information about the complex relationship not only between plant population size and the behaviour of pollinators, but also about the effects of associated flowering plants on the efficiency of this relationship.

Insects that confine their menu choice to one or few plant species are called specialists; insects foraging on several or many plant species are called generalists. These specialists are thought to be more sensitive to habitat deterioration than generalist insects, because interactions with their host plants will be lost (Olesen & Jain 1994) and the insects have no alternatives. The specialist bee species *Andrena marginata* forages exclusively on species of the family of Dipsacaceae (Westrich 1990), with *Succisa pratensis* as main pollen source and *Scabiosa columbaria* as one of the alternatives in the Netherlands (Peeters *et al*. 1999). Both plant species are decreasing rapidly in the Netherlands; *Succisa pratensis* is classified as a “sensitive species” (from >10,000 1x1 km squares in 1935 towards 1000-3000 in 1995; Tamis *et al*. 2004), and *S. columbaria* is considered as an “endangered species” (from 300-1000 1x1 km squares in 1935 towards 31-100 in 1995). The bee species used to be rather common in the eastern part of the Netherlands, but in 1962 it was observed for the last time (Peeters *et al*. 1999). Data of other insect species as flower visitors of the two plant species in the past are scarce. Knuth (1898) mentioned 37 insect species visiting *S. pratensis* in north and central Germany, with most species belonging to the Hymenoptera (40.5%). Proctor *et al*. (1996) especially mentioned *A. marginata* with *S. pratensis* as one of its favourites.

Flowers of many plant species are visited by more than one insect group and by even more insect species (see for instance Herrera 1987; Petanidou 1991; Waser *et al*. 1996; Johnson & Steiner 2000; Keys *et al*. 2005). Visiting insects may differ in the efficiency of pollen collection from the anthers and pollen deposition on the stigmas. An estimate of effectiveness of visitors for pollination of the whole plant population can be made from various perspectives (see also Waser & Price 1990): the number of visits spent by insect groups, and several foraging characteristics important for the efficiency of visitation of the two main groups, e.g. foraging speed (the number of flowers and flower heads visited per minute), and body pollen loads (the number of pollen grains on the insects). Flowers may also receive visits from insect species that visit other plant species (generalists) as well, and of insect species or individuals that confine their visits to one or a few related species (specialists) (Jordano 1987; Bronstein 1995; Waser *et al*. 1996; Memmott 1999; Olesen & Jordano 2002). Because flowers of many plant species are visited by a variety of insects, often including both generalists and specialists, the disappearance of a specialist insect does not need to be a catastrophe for the plant species under concern. However, the pollen load deposited on stigmas by specialists may contain more conspecific pollen grains than that deposited by generalist insects. Thus the quality of the pollination may change from a more or less pure stigmatic pollen load to a more heterospecific stigmatic load if the visitor guild changes from one containing an important contribution of specialists towards one with more generalists. Only a few studies include both pollinator abundance and effectiveness. These studies suggest that decoupling of pollinator abundance (quantity aspect) and pollinator quality occurs frequently among insect-pollinated plants (Sugden 1986; Schemske & Horvitz 1989; Pettersson 1991; Mayfield *et al*. 2001). Olsen (1997) found that pollinator importance, calculated as the product of pollination efficiency and relative abundance, was dictated by a pollinator's relative abundance. In his study, insects differed up to three times in pollination efficiency.

In this paper we analyse the effect of population size and the biotic environment, i.e. a low or a high diversity of other flowering plants, on visitor guild, visitation rate and pollen deposition in populations of *Succisa pratensis*. We assume that in a large population insects will be more flower-constant, either passive or active, so stigmatic loads in large *S. pratensis* populations will contain more conspecific pollen grains than in small populations. A diverse environment invites insects to visit more plant species during a single foraging trip. Furthermore, we relate frequencies of insect species and visitation rates to the number of conspecific pollen on the stigmas.

MATERIAL AND METHODS

The plant

Succisa pratensis Moench (devil’s bit scabious, Dipsacaceae), a perennial herb, grows occasionally in unfertilised hay fields and along roadsides in the Netherlands. It is a characteristic species of biodiverse, slightly acidic grasslands (Nardetea) (Vergeer *et al*. 2003b). Changes in land use, habitat fragmentation and deterioration have reduced its distribution area by 50-75% during recent decades (van der Meijden *et al*. 2000). Vergeer *et al*. (2003a) showed that population size is indeed strongly influenced by habitat quality. The remaining populations are isolated from each other and many are very small. The main flowering season in the study area starts in August and continues till mid October. The plant forms 1-10 flower branches, with 1-20 flower heads each. The blue-violet flowers (3 mm long) are arranged in hemispheric flower heads, with on average 60 flowers per head (diameter 2-3 cm). The flowers are protandrous, first presenting four anthers (in a sequence of two groups of two). The first flowers are displayed in two crowns at the bottom and near the top of the flower head. Both anthers and styles protrude out of the small, tubular flower. Both male and female flowers produce nectar at a rate during day time of 0.029 μl per hour with a sugar concentration of 14.1% (M.M. Kwak, unpubl. data).

Populations

Data were collected in nine populations in the north of the Netherlands in 2002 and 2003. Five populations were located in road verges near Assen (De Haar, Gasteren, Ekehaar, Eleveld and Annen, in the province of Drenthe), three populations in nature reserves (Wijnjewoude and Rotstergaast, in the province of Fryslân; and Reitma in the province of Drenthe). One population was artificially created in 1991 (Assen). In this chapter only data collected in the month September are presented to minimise the variation in phenology of insects and plants. *Succisa* populations differed in number of flowering heads (called population size) and the number of simultaneously flowering co-occurring plants. On each observation day the total number of flowering heads at that moment was counted and used as population size. Population characteristics are mentioned in table 7.1.

Insect observations

SPECIES FREQUENCIES AND VISITATION RATE

Insect observations were made in 2002 and 2003, between 10.00 and 16.00 h. In each population once per observation day, the number of individuals per species of insects visiting *S. pratensis* flowers were counted in a transect of ca. 100m length, depending the population size. In addition, in a plot with a known number of flower heads (35), insect visits were scored during ten minutes. This was done between two and four times per day per population.

FLOWER CONSTANCY: VISITATION SEQUENCES

The flower constancy of three syrphid species that visited *Succisa* flower heads most frequently was analysed (in 2002). Flower constancy is a quality component of pollination (when an insects are highly constant, then a pure pollen deposition load is expected). Following of insects was done in three populations: Ekehaar, Wijnjewoude and Annen. Observations always started with a visit to a *S. pratensis* head. The amount of changing was calculated by dividing the number of intraspecific transitions by the total number of transitions. This index ranges from 0 to 1 and the outcome is the proportion of intraspecific transitions (Slaa & Biesmeijer 2003). The minimum bout length for analysing was 5 visits, which equals four transitions.

FLOWER CONSTANCY: BODY POLLEN-LOAD COMPOSITION

Flower constancy over a longer period of time can be derived from the composition of the pollen load on the body of insects. Although syrphid flies spend time in cleaning their body and eating pollen, they often carry a reasonable amount of pollen on their body. In order to detect differences in flower constancy between four common syrphid species, pollen body loads were analysed. Ten individuals per species were sampled. Insects were captured after the observation that they had visited a head of *S. pratensis*; resampling was prevented. Insects were slightly anaesthetised with CO_2 and the ventral side of the body (this parts may contact *S. pratensis* stigmas) was cleaned with a piece of a sticky gel (Beattie 1972). A microscope slide was made by melting the gel and at least 300 pollen grains

Table. 7.1. Characteristics of the *Succisa pratensis* populations under investigation. Population size is given between brackets as maximum of the counted number of flowering heads during the observation period. Number of simultaneously flowering plant species is given between brackets. Observations are made on 1. frequencies of visiting insects and visitation rates; 2. flower constancy; 3. composition of pollen loads on insect bodies; 4. composition of stigmatic loads; 5. number of *S. pratensis* pollen grains per stigma. Observation 1 is made in 2002 and 2003, 2 and 3 only in 2002, 4 and 5 only 2003.

Population	coordinates	maximum population size	Number of simultaneously flowering plant species	observations
De Haar	52°58'N, 6°32'E	small (135)	low (5)	1
Eleveld	52°57'N, 6°34'E	small (160)	low (7)	1, 4, 5
Gasteren	52°02'N, 6°40'E	small (250)	low (4)	1, 3, 4, 5
Ekehaar	52°56'N, 6°37'E	small (350)	high (17)	1, 2, 3
Reitma	52°53'N, 6°40'E	large (6000)	low (2-3)	1, 4, 5
Wijnjewoude	52°03'N, 6°10'E	large (>10,000)	low (2)	1, 2, 3
Rotstergaast	52°55'N, 5°56'E	large (27,000)	low (3)	1
Annen	53°04'N, 6°41'E	large (1,600)	high (9-16)	1, 2, 3, 4, 5
Assen	52°59'N, 6°35'E	large (1,750)	high (30)	1

were counted and identified under a light microscope (10 x 10 or 10 x 40 magnification). A reference pollen collection of flowering species in the environment was prepared in the same way. In order to detect effects of population size and diversity of the flowering environment on the composition of the body load, two syrphid species were sampled in four populations.

Plant observations

POLLEN COMPOSITION OF STIGMATIC LOADS

The overall result of foraging of all insects is pollen deposition on stigmas. Before the start of the observations, heads were checked with a hand magnifier of 15x for virginity and receptivity (only in 2003). Heads were allowed to be visited during 5-6 hours during the day. After this period, stigmas (from seven heads per population, five populations) were cleaned and microscope slides were prepared in the same way as was done with the insects. If possible, 300 pollen grains were counted and identified; the percentage of *S. pratensis* was calculated.

NUMBER OF *S. PRATENSIS* POLLEN PER STIGMA

Virgin female heads that will become receptive that day were marked before observations started (only in 2003). At the end of the day (an exposure period of 5-6 hours) the number of pollen grains per stigma (n= 15 per head) was counted with a magnifier. Seven heads per population were counted. For seed set not only the mean number but also the distribution of the grains over the stigmas is important. Often a surplus of pollen is needed for the fertilisation of one ovule (Waser & Fugate 1986). We used the same minimum number of grains as is needed in the related species *Scabiosa columbaria* (Velterop 2000): four. We calculated the potential seed set: the percentage of flowers per flower head that had more than four pollen grains per stigma.

Statistics

The effect of population size and flower species richness on the number of visitor species, purity of pollen deposition on stigmas, the number of deposited pollen and potential seed set were analysed with multiple linear regression followed by a simple curve-fit analysis. Changing indices of insects were tested for significance within and between populations with Kruskal-Wallis tests, with Mann-Whitney tests for differences between groups. Body loads of insects were tested for significance within and between populations with univariate analyses of variance (ANOVA) with Tukey-tests as post hoc test for differences between groups. The effect of population type on visitation rate, purity of pollen deposition on stigmas, the number of deposited pollen and potential seed set were analysed with univariate ANOVA with Tukey-tests as post hoc test for differences between groups. All statistical tests were performed with the package SPSS for Windows version 12.0.1 (2003).

RESULTS

Insect observations

SPECIES FREQUENCIES AND VISITATION RATE

In 2002, 21 insect species visited *S. pratensis* (transect observations, fig. 7.1); the large majority of visits were from syrphid flies (96.2%). In 2003, 23 species were observed (plot observations), but the proportion of syrphid species and visits (53.9%) was lower than in 2003, and those of bees (almost exclusively bumblebees) was higher (32.7%, fig. 7.1). The contribution of insect species per population and per day to reach 75% of all visits was calculated and the frequency of being one of the contributors was scored. The frequency of these scores is shown in figure 7.2. Twelve species contributed to 75% of the visits on at least one day in one population. *Eristalis tenax, H. trivittatus* and *B. pascuorum* can be considered as the most important visitors for *S. pratensis*. The number of species contributing to 75% was larger in 2003 than in 2002 (10 vs. 7), and the years differ in bee and syrphid species.

The population size of *S. pratensis* affected the number of visitor species positively, while the flower diversity of the environment had no influence on the insect species composition of *S. pratensis*. The relation between *S. pratensis* population size as a continuous variable and the number of visitor species was best described with logarithmic function ($r^2 = 0.69$, fig. 7.3). There was no relation between flower diversity as a continuous variable and the number of visitor species.

In 2002, small populations had fewer visitor species than only one large population (11, 6 and 9 vs. 18 and 9) for all days combined (fig. 7.3). Small populations had *Eristalis horticola, E. tenax, Helophilus pendulus* and *H. trivittatus* as most frequently occurring

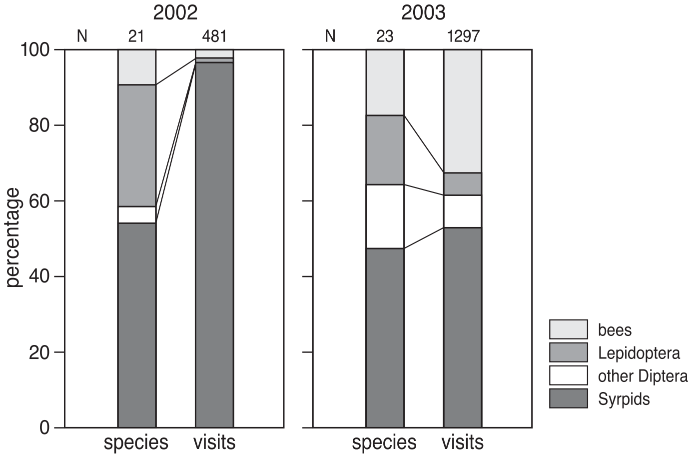


Fig. 7.1. Proportions of four main insect taxonomical groups as percentage of all species and the percentage visits brought by the insect groups for *Succisa pratensis* in 2002 and 2003. The N-values are the number of species (left bars within years) or visits (right bars). The lines between the bars indicate the difference of the relative contribution of the taxonomical groups to the number of species and the number of visits.

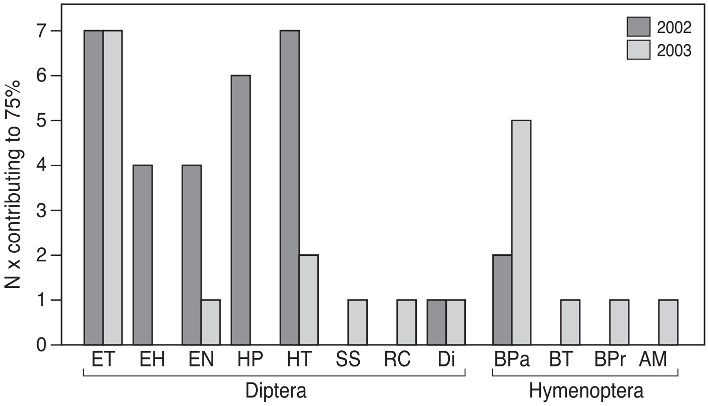


Fig. 7.2. Insect species contributing to 75% of visits on *Succisa pratensis* in 2002 and 2003. The contribution of insect species per population and per day to reach 75% of all visits was calculated, and the frequencies of these scores are shown as bars. ET= *Eristalis tenax*, EH= *E. horticola*, EN= *E. nemorum* and *E. arbustorum*, HP= *Helophilus pendulus*, HT= *H. trivittatus*, SS= *Sericomyia silentis*, RC= *Rhingia campestris*, Di= other Diptera, BPa= *Bombus pascuorum*, BT= *B. terrestris*, BPr= *B. pratorum*, and AM= *Apis mellifera*.

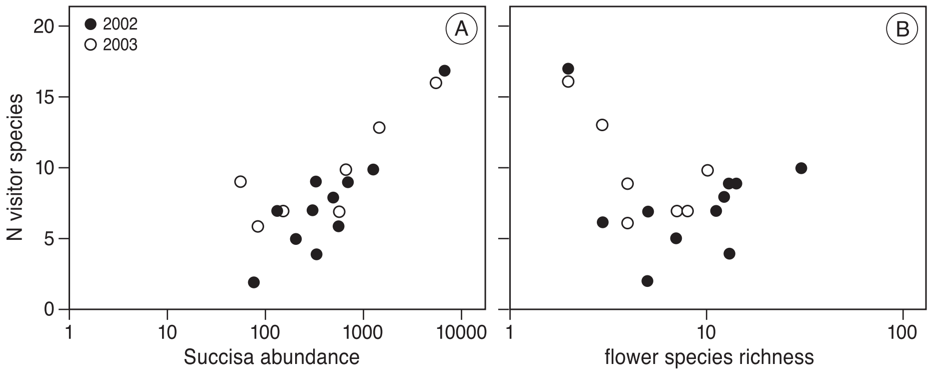


Fig. 7.3. Effect of the abundance of *S. pratensis* (A) and flower species richness (the number of other flowering species in the environment, B) on the number of flower visiting insect species on *Succisa pratensis*. There was a positive effect of population size ($r^2 = 0.69$), but no significant effect of flower species richness. Both x-axes are log-scaled. The data points are shown for the two years separately, but r^2-values are based on all data together.

species. Large populations had the same species, but also small *Eristalis* species (*E. nemorum* and *E. arbustorum*). In one small population (Ekehaar), *Bombus pascuorum* was an important visitor on one day. In 2003, the two small populations had fewer visiting species than the two large populations (10 and 7 vs. 20 and 14) for all days (fig. 7.3). The most important visitors in the small populations were bumblebees (*B. pascuorum*) and syrphids (*E. tenax*), in the two large populations mainly syrphids (*E. tenax*), and in the large population Reitma also bumblebees (*B. pascuorum*).

The mean number of visits received per *S. pratensis* flower head varied in 2002 between 5 and 13 visits per hour per day; in 2003 between 5 and 12. In both years the visitation rates did not differ significantly between population types either size or diversity of the environment (2002: $F_{3,33} = 2.4$, $p > 0.05$; 2003: $F_{3,20} = 0.73$, $p > 0.5$; fig. 7.4).

FLOWER CONSTANCY: VISITATION SEQUENCES

The observed syrphid species showed a flower constancy between 0.74 and 1.0, which means that most of the visited flowers were *S. pratensis* (table 7.2). Only *Helophilus pendulus* did react significantly concerning population type (diversity of the environment): in the populations with a high flower diversity its flower constancy was lower than in populations with a low flower diversity ($X^2 = 13.3$, $p < 0.005$). *Helophilus pendulus* was more constant in a larger *S. pratensis* population with a low plant diversity (0.99), and it tended to be intermediate in the large population with a high flower diversity (0.86), but did not differ significantly from the small population (0.74). Flower constancy varied only for *H. pendulus* but not the other two syrphid species, that always had constancies larger than 0.90. The species differed significantly in population small-high ($X^2 = 6.9$, $p < 0.05$; table 7.2). Overall, the flower constancies of the three species are 0.86, 0.96 and 0.95 respectively (data derived from table 7.2).

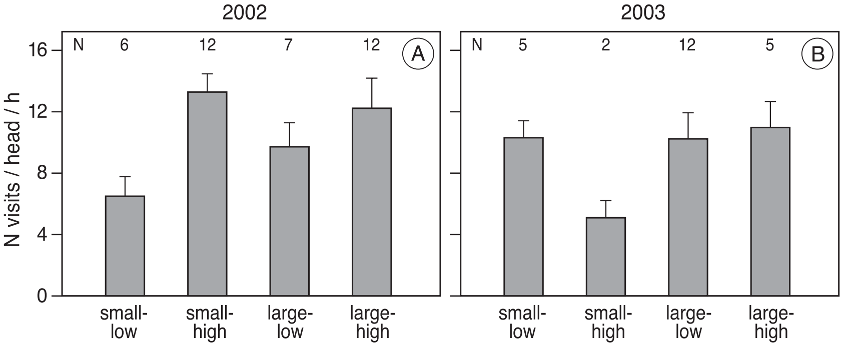


Fig. 7.4. Visitation rates on *Succisa pratensis* patches at four population types, based on flower abundance of *S. pratensis* (small or large populations) and the number of simultaneously flowering plant species (low or high diversity) in 2002 (A) and 2003 (B). No significant differences were found between populations types in any of the years.

Table. 7.2. Flower constancies (mean ± SE) of three syrphid species as frequent visitors of *Succisa pratensis* in populations differing in number of flowering heads of *S. pratensis* (small, large) and other flowering species (low, high). The populations were Ekehaar, Wijnjewoude and Annen. Flower constancy is the number of intraspecific transitions per plant species divided by the total number of transitions. The number of sampled individuals are between brackets. Significant differences between species are indicated with different capital letters, between the populations with small letters, n.a. means no value available.

	Population type (size *S. pratensis*, diversity flowering plants)		
Syrphid species	small, high	large, low	large, high
Helophilus pendulus	0.74 ± 0.09 (7)^{Aa}	0.99 ± 0.01 (18)^{b}	0.86 ± 0.06 (9)^{a}
Helophilus trivittatus	0.95 ± 0.03 (17)^{B}	1.0 ± 0 (4)	0.94 ± 0.03 (11)
Eristalis horticola	0.95 ± 0.02 (14)^{B}	n.a.	0.94 ± 0.03 (11)

FLOWER CONSTANCY: BODY POLLEN LOAD COMPOSITION

Syrphid flies carried loads that contained between 20 and 78% *S. pratensis* pollen (table 7.3), and between 24 and 508 *S. pratensis* pollen grains (table 7.4). Not all syrphid species could be sampled in all populations, making comparisons between species difficult. Differences between the species were found for the percentage of *Succisa* pollen at population large-low, where *H. pendulus* had a higher fraction *Succisa* pollen grains than the other species ($F_{3,36}$= 7.1, $p< 0.005$, table 7.3); for the absolute number of pollen in the populations small-high ($F_{1,18}$= 11.8, $p< 0.005$) and large-high ($F_{2,29}$= 18.3, $p< 0.001$), where *E. horticola* carried five to seven times more *S. pratensis* pollen grains than the other syrphid species (table 7.4).

Table. 7.3. Percentages of *Succisa pratensis* pollen grains relative to other plant species in body loads of four syrphid species in four *S. pratensis* populations. The number of sampled individuals are between brackets. Significant differences between species are indicated with different capital letters, between the populations with small letters, n.a. means no value available.

	population type (*S. pratensis* size, diversity of flowering plants)			
Syrphid species	small, low	small, high	large, low	large, high
Helophilus pendulus	23 ± 7 (10)^{a}	20 ± 5 (10)^{a}	78 ± 5 (10)^{Ab}	54 ± 9 (9)^{b}
Helophilus trivittatus	n.a.	n.a.	38 ± 10 (10)^{B}	40 ± 9 (11)
Eristalis horticola	n.a.	37 ± 7 (10)^{a}	45 ± 10 (10)^{Bab}	67 ± 8 (11)^{b}
Eristalis tenax	n.a.	n.a.	24 ± 9 (10)^{B}	n.a.

Table. 7.4. Number of Succisa pratensis pollen grains in body loads of four syrphid species in four S. pratensis populations. The number of sampled individuals are given between brackets. Significant differences between species are indicated with different capital letters, between the populations with small letters, n.a. means no value available.

	population type (*S. pratensis* size, diversity of flowering plants)			
Syrphid species	small, low	small, high	large, low	large, high
Helophilus pendulus	95 ± 51 (10)	49 ± 11(10)^{A}	66 ± 19 (10)	102 ± 27 (9)^{A}
Helophilus trivittatus	n.a.	n.a.	27 ± 6 (10)a	105 ± 24 (11)^{Ab}
Eristalis horticola	n.a.	341 ± 84 (10)Bab	96 ± 51 (10)a	508 ± 85 (11)^{Bb}
Eristalis tenax	n.a.	n.a.	24 ± 9 (10)	n.a.

The loads of *H. pendulus* were more pure in large than in small populations (large populations: 54-78 %; small populations 20-23 % *S. pratensis* pollen grains; $F_{3,35}$= 17.4, p< 0.001; table 7.3). For the absolute number of pollen grains there was a similar tendency, but this was not significant (table 7.4). Also the loads of *Eristalis horticola* contained a larger proportion of *S. pratensis* pollen grains in large than in small populations ($F_{2,28}$= 3.4, p< 0.05; table 7.4). However, the difference in absolute number of *S. pratensis* pollen grains does not appear to be related to population size (F2,28= 7.5, p< 0.005; table 7.4) . The absolute number of pollen grains differed also for *H. trivittatus* between the two populations it was sampled . The syrphid *E. tenax* could only be sampled at the site large-low.

Heterospecific pollen grains were from the types *Hieracium/ Hypochaeris/ Leontodon, Calluna/ Erica*, and *Achillea/ Tanacetum*, all species with pollen that could easily be picked up by the syrphids. Despite the fact that in the large *S. pratensis* population only one other species was in flower (*Potentilla erecta*), the loads of the insects contained between 22 and 80% heterospecific pollen grains. Most striking was the presence of pollen of *Erica/ Calluna*, species not present in the direct vicinity.

In summary, the average body load of syrphids contained respectively (from small and low diversity to large and high diversity of plants) 23, 29, 47 and 54 % *S. pratensis* pollen grains, and 95, 195, 53, and 238 absolute number of *S. pratensis* pollen grains (data derived from tables 7.3 & 7.4).

Plant observations

POLLEN COMPOSITION OF STIGMATIC LOADS

The mean proportion of conspecific (i.e. *S. pratensis*) pollen grains per flower head varied between 39 and 97% (fig. 7.5A). The proportion was positively related to population size and plant species richness (fig. 7.5D). In a curve-fit analysis, a logarithmic function gave the best description of the relation between population size and conspecific pollen (r^2= 0.47, $F_{1,58}$= 51.2, p< 0.001). The large/low population received a significantly higher proportion of *S. pratensis* pollen grains than the other populations ($F_{4,56}$= 19.2, p< 0.001=0.000, fig. 7.6A).

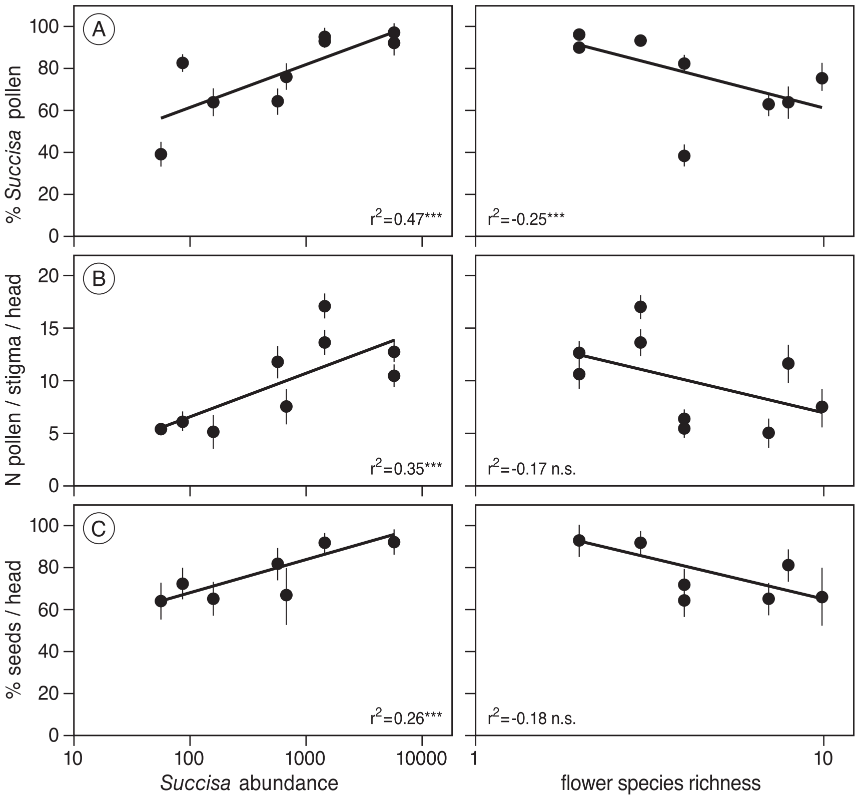


Fig. 7.5. Relation between *S. pratensis* "population size", i.e. flower abundance, or flower species richness (both log scaled) and the percentage of conspecific (*S. pratensis*) pollen on stigmas (A), the number of conspecific pollen grains deposited per stigma per head of *S. pratensis* (B) and potential seed set (C) in 2003. Potential seed set is based on a minimum of four pollen grains per stigma (see text).

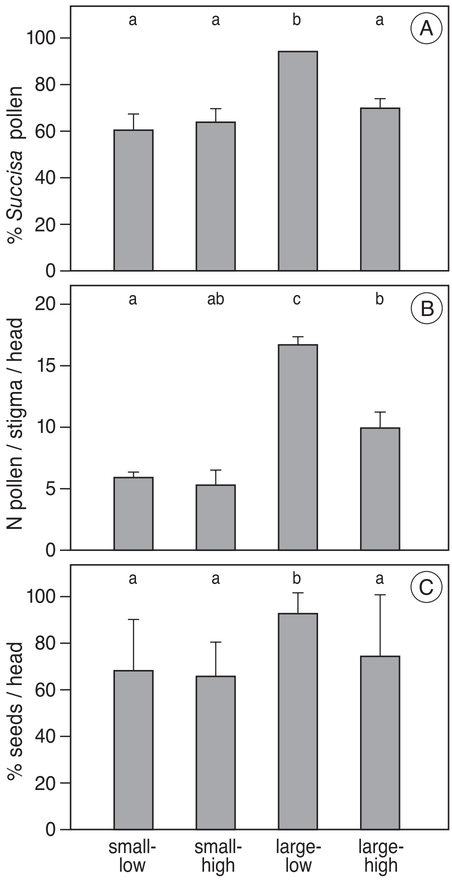


Fig. 7.6. Pollen composition of stigmatic loads in percentage of *Succisa pratensis* pollen (A), the number of conspecific pollen grains deposited per stigma per head of S. pratensis (B) and potential seed set (C) in populations different abundances of *S. pratensis* (small or large) and differing number of simultaneously flowering plant species (low or high) in 2003. Means are ±SE. Potential seed set is based on a minimum of four pollen grains per stigma (see text). Different letters indicate significant differences between populations.

NUMBER OF *SUCCISA* POLLEN PER STIGMA

The mean number of *S. pratensis* pollen grains per stigma varied between 5 and 17 per stigma (fig. 7.5B). The number of pollen grains per stigma was positively related to population size, and negative, but less strongly, to plant species richness (fig. 7.5D). In a curve-fit analysis, logarithmic functions gave the best description of the relations (size: $r^2 = 0.35$, $F_{1,58} = 31.0$, $p < 0.001$; diversity: $r^2 = 0.25$, $F_{1,58} = 19.5$, $p < 0.001$). Generally, large populations had a larger number of *S. pratensis* pollen per stigma than small populations ($F_{4,56} = 18.8$, $p < 0.001$; fig. 7.5B). The large/low population received a significantly higher proportion of *S. pratensis* pollen grains than the other populations (fig. 7.6B).

POTENTIAL SEED SET

The mean percentage of flowers per head that potentially set seed varied between 65 and 96% (fig. 7.5C). The number of pollen grains per stigma was positively related to population size, but not plant species richness (fig. 7.5F). In a curve-fit analysis, a logarithmic function gave the best description of the relation between population size and the number of deposited pollen grains per stigma per head ($r^2 = 0.26$, $F_{1,58} = 20.3$, $p < 0.001$). Potential seed set was highest in the large population with a low plant diversity ($F_{4,56} = 8.4$, $p < 0.001$; fig 7.6C).

DISCUSSION

Insect species

Succisa pratensis populations in the Netherlands differed in the composition of the visitor guilds. Between 5 and 20 insect species per population were observed; in total (2002 and 2003 combined) 34 insect species were visitors of *S. pratensis*. In 2002, bumblebees were nearly absent: only 1.4% of the total number of visits were made by bumblebees. In 2003, bumblebees were responsible for 29.5% of the visits with locally more than 50%. These data illustrate that the composition of the visitor guilds may vary largely between populations and years; population size may have an effect but variation in flower visitor guilds is normal. Variation in flower-visiting insects was also found by various other authors (Herrera 1988, 1989 & 1995; Ashman & Stanton 1991; Eckhart 1995; Ramsey 1995; Fishbein & Venable 1996; Herrera 1996; Kato 1996; Traveset & Sáez 1997; Comba *et al*. 1999; Memmott 1999).

We never observed the bee species *Andrena marginata*. Knuth (1898) mentioned this bee species for *Knautia arvensis* (observations by Alfken and Höppner near Bremen, Germany), together with many other insect species, 57 species in total. Also Proctor *et al*. (1996) mentioned this bee species for *S. pratensis*. Knuth (1898) mentioned that bee species were up to 41% (solitary bees and bumblebees, but not *A. marginata*) and syrphids only 27% of the visiting species of *S. pratensis*. Adams (1955) observed 15 bee species and only three fly species on *S. pratensis* in Great Britain. Willis & Burkill (1895) presented 58 insect visitors of *S. pratensis*, but *A. marginata* was not among the Hymenoptera mentioned. Also in French *Succisa* populations (Vosges, Lorraine), *A. marginata* was not found (2 populations were studied in 2003, unpubl. data) and syrphids were the dominant flower visitors (between 50 and 95% of visits, and 51% of the species, N= 29 insect species). Bees, in total, brought up to 30% of the visits in one population, including small solitary bees (but not *A. marginata*).

Succisa pratensis is well known as food source for butterflies. Knuth (1898) mentioned 7 species of Lepidoptera, Jennersten (1984) mentioned four species that were responsible for 40% of the visits to *S. pratensis* heads. We observed six butterfly species (31.8% of all species) responsible for only 1.8% of the visits in 2002, and four butterfly species (17.4% of all species) responsible for 5.2% of the visits in 2003. The late flowering time may be one of the reasons for the absence of butterflies as visitors. In general the frequency of visits of butterflies is low compared to other insect species visiting the same plant species. In addition, the number of pollen grains deposited on the stigma by butterflies is restricted (Jennersten 1984; Velterop 2000), with the exception of a few plant species in the northwest European flora.

The bulk of the flower visits to *S. pratensis* in the Netherlands was made by a restricted number of insect species, mainly syrphid species and the bumblebee *Bombus pascuorum*. In total for all populations together, in 2002 we found five syrphid species as the most frequent visitors: *E. horticola*, *H. trivittatus*, *H. pendulus*, *E. tenax* and *E. arbustorum/ nemorum* in order of decreasing abundance. In 2003, *E. tenax*, *H. trivittatus* and *B. pascuorum* were the most frequent species on *S. pratensis* (figure 7.2). The behaviour of these frequently occurring insect species determines pollination quantity and quality aspects of *S. pratensis*.

Small populations were visited by fewer insect species than large populations. In addition, small populations had sometimes bumblebees as most frequent visitor; in one large population bumblebees were the second frequent visitor. Taking into account not only the frequency of individuals, for instance the ratio between bumblebee and syrphids, but also *Succisa* pollen deposition (ratio bumblebees: syrphids = 1 : 0.7) the importance of bumblebees in relation to the number of deposited pollen increases (Kwak 1993). We expected that populations with many bumblebee visits should have a higher *Succisa* pollen deposition compared to heads in populations with the same visitation rates but visited by syrphids. Indeed, this expectation came true for the large populations in 2003.

Flower constancy in relation to the purity of the stigma pollen load

The behaviours of the most frequent insect species, syrphids in 2002, determined the pollen load on the stigmas. The flower constancy of syrphids is influenced by the population size of *S. pratensis* and the presence of co-occurring and simultaneously flowering plant species. Syrphid species differed in their flower constancy, measured simultaneously in one population, with *H. pendulus* being a less flower-constant species compared to *H. trivittatus* and *E. horticola*. Although individual syrphid species may be constant for the time that they were observed, they may carry a body pollen load with pollen grains of plant species not occurring in the direct vicinity. The load reflects the plant species that were visited during a longer period, preceding the foraging in a *S. pratensis* population. We found *Erica* and *Calluna* pollen, and the nearest population of these plant species is at least 300 m away from the *S. pratensis* population.

Population size of *S. pratensis* determines the purity of the body loads of insects. In small *S. pratensis* populations the average syrphid load contained between 23 and 18.5% *S. pratensis* pollen and in large populations between 46.5 and 54%, a clear effect of *S. pratensis* population size. The effect of the flowering environment is reflected in more heterospecific pollen deposition in a diverse environment, but only for *H. pendulus* regardless of the population size of *S. pratensis*. For the percentages of *S. pratensis* pollen on the stigmas we see the same trend: less conspecific pollen in smaller (around 60%) than in larger populations (70 and 100%).

Visitation rate and number of *S. pratensis* pollen per stigma and potential seed set

The visitation rate varied in 2002 between 5 and 17, in 2003 between 5 and 18 visits per *S. pratensis* head per hour. That means that every 3 to 12 minutes an insect landed on a *S. pratensis* flower head; this high visitation rate of *S. pratensis* led to the following comparison made by Lack (in Proctor *et al*. 1996): '…the number of visitors around each inflorescence may be so large that the scene resembles aircrafts stacked in the air waiting to land at a too-busy airport.' Assuming a visitation day of six hours, each head will have received between 30 and 108 visits. These values seem sufficient for complete pollination. However, to fulfil both the female and the male function of the flower (the flower head is in the male phase between 6-8 days, the female phase usually for only 1 day), also heads in the male phase need to be visited. One visit per day to a female head is not enough to pollinate all stigmas. To achieve full seed set, heads must be visited several times during the receptive female phase, which generally lasts only a single day. Each visitor spent such a short period per head, that this is too short to deposit at least four grains per stigma in all flowers of a head (Kwak 1993). Stigmas had received between 5 and 17 pollen grains at the end of the day (in 2003), but small populations had received significantly less conspecific pollen than stigmas in large populations. Potential seed set was high, and varied between 65 and 94% of the flowers per head per population. Small populations still do receive many insect visits, but the amount of available pollen in such small populations may be restricted. For instance, in 2003 the visitation rate in the small population Gasteren was around the same as in the large populations Reitma and Annen (10 visits per head per hour), but the resulting pollen deposition was respectively 6, 17 and 10 pollen grains per stigma. Thus, not only visitation rate is important for pollination, but also the number of conspecific pollen grains deposited. In small populations, flower density of the target species may be low and visitors may have lost the conspecific pollen before arriving on a *S. pratensis* head, for instance due to visits to other plant species.

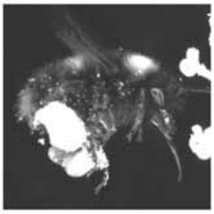
In conclusion

Large populations of *S. pratensis* are visited by more insect species, but flowers in large *S. pratensis* populations do not receive more visits per head than flowers in small populations. However, *S. pratensis* flowers in large populations have a larger conspecific pollen load on the stigmas with the expectation that the potential seed set is also larger in large populations. The diversity of the environment, e.g. the number of co-occurring, simultaneously flowering plants, has no influence on the purity of the stigmatic pollen load: insects may forage for short periods on only one plant species (flower constant). However, they often carry a mixed body load sometimes containing pollen grains of plant species not occurring in the immediate vicinity.

Most of the data point in the same direction: there is no difference in visitation rates between small and large populations but the quality of pollination (number of *Succisa* pollen grains, purity of the stigmatic load, distribution of the pollen grains over the stigmas within a head) is more favourable for seed set in large than in small populations, with a strong effect of population size and much less of the diversity of the environment. This effect of diversity of the environment is pronounced in small *S. pratensis* populations. If heterospecific pollen deposition leads to a lower seed set, then small populations in a diverse environment will have the lowest percentage of viable seeds per head and large populations in an environment with no other simultaneously flowering species will have the highest percentage of seed set. To date it is not known whether heterospecific pollen have a negative effect on the fertilisation of *S. pratensis*. The diversity of insects did not really play a role in the investigated populations. Only a few insect species are responsible for the bulk of the visits, but they all may visit also other plant species, resulting in heterospecific pollen deposition.

The bad quality of pollination in small *S. pratensis* populations decreases the reproduction, thereby increasing the extinction chance of these small populations. The presence of a specialist forager may increase the pollination quality. For *S. pratensis* this would be the bee *Andrena marginata*, but as we already know, it is extinct in the Netherlands now. The missing link of knowledge is the pollination quality of this bee: if it was better than syrphids and bumblebees, then the shift to a more generalist pollinator assemblage of only syrphids and bumblebees had negative consequences for the pollination of *S. pratensis*. On the other hand, it may be that even though *A. marginata* is a better pollinator than the other species, in large *S. pratensis* it does not matter as the pollination is also suffient with only generalist pollinators. Furthermore, small *S. pratensis* populations may be too small for sustaining *A. marginata* populations, and thus cannot benefit from this bee. Thus in the end there may be a positive feedback of declining plant populations affecting the abundance and the combined efficiency of all pollinator species, thereby speeding up the decline of the plant population. The presence and abundance of specialist pollinators therefore can be faster indicators of declining plant population size. Solitary bees are more affected by landscape deterioration than many other insect groups (chapter 3) and more than 50% of all bee species in the Netherlands are red-listed (Peeters & Reemer 2003). For many plant species this may be a "bad omen" if the same happens them as to *S. pratensis*, whose bee had disappeared long before the plant showed the steep decline.

Finally, the data presented in this chapter show how true the sentence of Fægri & van der Pijl (1979) is about flower visiting insects: ‘What they do today and in a particular place is not necessarily the same what they will do tomorrow and in another place'.



8

Flower visitation of a plant community: just a few visitor species for many plants, but how many pollinators?

Frank Hoffmann

SUMMARY

For 60 plant species in a plant community in the Netherlands I have investigated whether flowers of most species are visited by a small set of insect species, and whether those insects are sufficient for the pollination of all plant species in that community. Only 10 insect species (2.5% of 397 observed) constituted more than 50% of the visitors of 75% of the plant species. These were three fly, four syrphid, two bumblebee species and the honeybee. These are all very abundant, common and generalist insects, occurring during most of the season. The higher the level of specialisation of a plant species, the larger was the proportion of the ten most frequent insect species. For 50% and 75% of the visitors in all 60 plant species, 39 (9.5%) and 93 (18.2%) of the observed insect species were needed, respectively. For a guaranteed pollination of the entire plant community the ten most frequent insects are not enough: some of the "top-ten" visitors are low-quality pollinators, and more pollinator species are needed for the pollination of the plant community for reasons involving degree of specialisation, spatio-temporal variation, plant population structure and interactions between plants. For a sustainable minimal pollinator fauna, a good deal of habitat heterogeneity, and a continuous and diverse food supply (flowers) for the insects throughout the flowering season are needed.

INTRODUCTION

Insects that confine their menu choice to one or few plant species are called specialists; insects foraging on several or many plant species are called generalists. Similarly, plant species with many pollinator species or taxa are generalists, and plants with few pollinators are specialists. The majority of plant-pollinator interactions is of a generalised nature (Jordano 1987; Ellis & Ellis-Adam 1993; Waser *et al*. 1996; Memmott 1999; Ollerton & Cranmer 2002). The distribution of interactions between plants and flower visitors is highly asymmetrical (Jordano 1987; Bronstein 1995; Waser *et al*. 1996; Memmott *et al*. 2000; Olesen *et al*. 2002). This means that specialist plant species are mainly visited by generalist insects, whereas many specialist insects visit generalist plants. For many plants this may cause resilience to pollinator species loss, as pollinators that have disappeared may be compensated by other present pollinator species (Rathcke & Jules 1993; Waser *et al*. 1996; Spira 2001).

Plant-pollinator communities are also stabilised by highly linked species in a network (Jordano 1987; Corbet 2000b; Olesen & Jordano 2002; Bascompte *et al*. 2003; Jordano *et al*. 2003; Memmott *et al*. 2004), as they are connectors between different compartments of that network (Dicks *et al*. 2002). These highly linked species are often very abundant, and in fact, most plant-pollinator communities are dominated by small groups of abundant plant and animal species (Memmott 1999; Dicks *et al*. 2002; Olesen & Jordano 2002). Species that interact with many more species than the other species in the community are sometimes called super- generalists (Olesen *et al*. 2002; Jordano *et al*. 2003). Because specialisation and generalisation are continuous across species (Armbuster *et al*. 2000; Johnson & Steiner 2000), super-generalists do not necessarily have a sharp boundary with other species (chapter 4). They are most distinctive in small and isolated habitats like oceanic islands, where species-area relations differ from mainland habitats (Olesen *et al*. 2002; Dupont *et al*. 2003).

The dominant species functioning as "interaction nodes" in a plant-pollinator network may also be considered as keystone species (Memmott 1999; Dupont *et al*. 2003), a concept that has not been widely applied to pollinator communities yet (Memmott 1999). Originally, the keystone-species concept was used for carnivores as mediators of top-down regulation of herbivores (Paine 1969). Since then it has also been applied to other trophic levels, but usually for species which play a disproportionally large role compared to their abundance (Power *et al*. 1996), but the discussion about the use and definition of keystone species has not been settled yet (Davic 2003).

Whether the dominant visitors are called keystone species, super-generalists or something else, in any case only few studies exist to show their relative importance (Memmott *et al*. 2004). Two questions are dealt with in this chapter: (1) Are most plant species in a community visited by a small set of insect species? (2) Are those insect species responsible for the pollination of the majority of the plant species? For the plant-flower-visitor community from chapter 2, I will identify the insect species that are the most frequent and potentially most important for pollination. I will then analyse whether those insect species contribute to a substantial part of the visitors of all plant species in the community, and whether this contribution is related to the degree of specialisation and flower type of a plant species. Finally, I will discuss whether these species are sufficient for sustainable pollination of the whole plant community, and how many other pollinator species may be needed.

MATERIAL & METHODS AND RESULTS

The potentially most important visitor species

The most frequently occurring (or potentially most important) insect visitor species are determined for a plant community in the north of the Netherlands. The data are from investigations of road verges and ditch banks situated in agricultural and semi-natural surroundings and collected in 2000 and 2001 (chapter 2, see there for a description of field methods). For analysis, plant species with at least 20 visitors (individuals) were used (60 species). From the insects observed to visit the flowers of these plants (397 insect species), the potentially most important visitor species were estimated, based on five criteria:

1. Abundance: the total number of observed individuals per insect species.
2. Animal linkage level (L_a): the total number of plant species from the sixty selected plant species an insect species was observed on.
3. N x in f_{10}: the number of plant species for which an insect species belonged to the ten most frequent visitor species.
4. N x in 50%: the number of plant species for which an insect species contributed to 50% of the visitors. For this purpose, the insects were sorted in order of frequency for each plant species. The most frequent insect species contributing to at least 50% of the visitors were selected.
5. N x in 75%: the same as 4, but then with at least 75% of the visitors.

Per criterium described above, the insect species with the highest scores were selected. The potentially most important insect species are those that are in at least three categories (i.e. in the majority of the five categories) among the ten species with the highest scores (table 8.1): three fly species, four syrphid species, two bumblebee species and the honeybee. Thus ten in total, therefore I will call them the "top ten" of visitors now.

Are the top-ten species enough for 50% and 75% of the visitors for all plant species?

For almost two third of the 60 plant species the top-ten visitors contributed to the majority of the visitors: in 18 out of the 60 plant species the top-ten species contributed to more than 75% of the observed visitor individuals, and in 19 plant species, the top-ten species contributed to between 50% and up to 75% of the observed visitor individuals. In 23 plant species, the top ten of insect species contributed to less than or equal to 50% of the visitors (table 8.2).

How many insect species are needed to have more than half and more than three quarters of the visitors in all plant species? To answer this, I calculated for each of the 60 plant species the number of insect species contributing to 50% or 75% of the visitors

Table. 8.1. Identification of the potentially most important insect visitors for 60 plant species based on five criteria (see text for explanations). All insect species belonging to the ten species with the highest scores in at least one category are listed. In each category the values of species with the highest scores are in bold. Insect species that are most often among the species with highest scores (last column) are the "top ten" species. The top ten species are listed in taxonomical order.

		Criteria					
Insect species	Functional group	Abundance	L_a	N x in f_{10}	N x in 50%	N x in 75%	N x highest10
Musca species[1]	fly	**5273**	**42**	**35**	**22**	**30**	**5**
little brown fly[2]	fly	**2309**	**31**	**25**	**13**	**22**	**5**
little black fly[2]	fly	**1949**	**31**	**18**	**11**	**17**	**5**
Eristalis tenax	syrphid	**1680**	**32**	**26**	**11**	**20**	**5**
Eristalis arbustorum[3]	syrphid	672	33	**28**	**8**	**22**	**3**
Episyrphus balteatus	syrphid	626	**35**	**19**	4	**15**	**3**
Rhingia campestris	Rhingia5	515	**37**	**24**	**8**	**18**	**4**
Bombus pascuorum	bumblebee	**959**	**41**	**30**	**16**	**24**	**5**
Bombus terrestris[4]	bumblebee	693	**45**	**28**	**10**	**20**	**4**
Apis mellifera	honeybee	**2127**	**34**	**22**	**11**	**18**	**5**
Bibio species	other diptera	**6098**	12	7	**5**	8	2
Sepsis species	other diptera	**1213**	11	2	0	1	1
Scathophaga stercoraria	fly	**1304**	25	13	3	9	1
Rhagonycha fulva	beetle	**1280**	18	8	1	6	1
Helophilus pendulus	syrphid	137	**34**	6	2	3	1

[1] *Musca* is a group of species that are difficult or impossible to distinguish in the field. [2] The "little brown fly" and "little black fly" are groups of similar species (morpho-species) of 4-8 mm length, beloning to Tachinidae and Muscidae. [3] *Eristalis arbustorum* includes the less abundant *E. abusivia*; the two species cannot be distinguished without catching every individual. [4] *Bombus terrestris* includes the less abundant *B. lucorum*, the two species cannot be distinguished without catching every individual. [5] *Rhingia campestris* is a long-tongued syrphid and therefore a different functional group (see chapter 2).

(individuals). The minimal number of insect species needed for 50% of the visitors of each of the 60 plant species was 39, for 75% it was 93 (table 8.3). This means that 9.5% (39 out of 397) of the observed insect species contributed to 50% of the visitors of the 60 plant species, and 18.2% of the insect species contributed to 75% of the visitors. Flies and syrphids have the highest numbers of species contributing to 50% of the visitors, respectively 10 and 11 (table 8.3). The most numerous species groups for 75% of the species are flies (19 species), syrphids (20) and solitary bees (16). Syrphids and wasps (particularly non-aculeates) have the highest number of species among the remaining 25% of the visitors (table 8.3).

Degree of specialisation and flower types

The plant species were subdivided into nine flower types (see chapter 2). The level of specialisation can be expressed as plant linkage level (L_p), i.e. the number of insect species

Table. 8.2. Plant species data: full names, short name, plant family, flower type (see chapter 2), known or assumed pollinators, total linkage level (L_p), and the number of insect species for 50% or 75% of the visitor frequencies. The main pollinators are based on unpublished data, chapter 6, and literature sources (Fægri & van der Pijl 1979; Kwak 1980; Kwak & Jennersten 1986; Kwak 1993; Ågren 1996; Kwak *et al*. 1998; van der Muren *et al*. 2003; Hunneman *et al*. 2004). For apomictic species no pollinators are indicated.

Plant family	Plant species	Flower type	known or assumed main pollinators	L_p[1]	N top 10 species	% top 10[2]	N 50%[3]	N 75%[4]
Apiaceae	*Aegopodium podagraria*	umbel	*Musca, Scathophaga, Eristalis*, Tachinidae, beetles	88	5	34	5	16
	Angelica sylvestris	umbel	*Musca, Eristalis, Myatropa florea*, Tachinidae, wasps	105	5	32	3	10
	Anthriscus sylvestris	umbel	*Musca, Empis livida, E. tessellata, Eristalis*, Tachinidae, beetles	120	3	27	5	9
	Heracleum sphondylium	umbel	*Musca, Tachinidae, Scathophaga, Eristalis*	119	4	19	2	4
Asteraceae	*Achillea millefolium*	umbel-head		67	3	27	6	16
	Bellis perennis	head		7	4	43	3	4
	Cirsium arvense	head		61	5	56	2	6
	Cirsium palustre	head	*Bombus, Eristalis, Helophilus*	24	5	56	6	25
	Crepis capillaris	head	[apomictic]	46	5	80	1	4
	Eupatorium cannabinum	brush	*Eristalis tenax, E. horticola, E. arbustorum, Musca*, *Bombus* spec.	59	3	56	3	5
	Hieracium aurantiacum	head	[apomictic?]	7	3	75	1	2
	Hieracium laevigatum	head	[apomictic]	103	9	66	5	14
	Hieracium pilosella	head	[apomictic]	48	3	19	8	20
	Hieracium umbellatum	head	[apomictic]	39	7	71	4	10
	Hypochaeris radicata	head	*Eristalis tenax, E. horticola, Panurgus, Dasypoda hirtipes, Helophilus, Bombus terrestris, Apis mellifera*	92	6	58	4	11
	Leontodon autumnalis	head	*Eristalis tenax, Panurgus, Muscidae*	49	7	84	2	5
	Matricaria spec	head		31	6	69	3	8
	Senecio jacobaea	umbel-head		17	4	50	3	7
	Sonchus arvensis	head		25	5	71	1	11
	Tanacetum vulgare	umbel-head		82	5	75	2	6
	Taraxacum cf. officinale	head	[apomictic]	59	6	32	3	9
Boraginaceae	*Symphytum officinale*	tube	*Bombus pascuorum, B. hortorum, B. pratorum?*	22	5	84	1	2

Table. 8.2. Continued

Plant family	Plant species	Flower type	known or assumed main pollinators	L_p[1]	N top 10 species	% top 10[2]	N 50%[3]	N 75%[4]
Brassicaceae	*Cardamine pratensis*	dish		23	2	32	3	7
	Rorippa amphibia	dish		17	4	27	4	7
	Rorippa nasturtium-aquaticum	dish		15	2	45	3	5
Campanulaceae	*Campanula rotundifolia*	bell	*Melitta haemorrhoidalis*	11	5	40	2	4
	Jasione montana	brush	*Eristalis intricarius, E. horticola*, *Bombus pascuorum*, . *B. pratorum*, div. solitary bees, *Helophilus* spec	40	5	34	8	22
	Phyteuma spicatum ssp. *nigrum*	brush	*B. jonellus, B. pascuorum, B. hortorum, Andrena*?	7	2	63	1	2
Caryophyllaceae	*Lychnis flos-cuculi*	tube		14	4	76	2	4
	Silene dioica	tube	*Bombus pascuorum, lepidoptera*?	14	3	69	1	4
Clusiaceae	*Hypericum perforatum*	dish	[apomictic]	29	9	82	5	9
Dipsacaceae	*Succisa pratensis*	head	*Eristalis tenax*, *Bombus pascuorum*	13	7	95	1	2
Ericaceae	*Calluna vulgaris*	brush	Apis mellifera, *Bombus*, solitary bees, Diptera	24	5	96	1	1
Fabaceae	*Lotus corniculatus*	flag	*Bombus pascuorum, B. lapidarius*	7	3	70	1	2
	Lupinus polyphyllus	flag	*Bombus*	5	2	89	1	1
	Trifolium pratense	flag	*Bombus hortorum, B. pascuorum*	14	3	66	1	3
	Trifolium repens	flag	*Bombus*, *Apis mellifera*	10	3	90	1	3
	Vicia cracca	flag	*Bombus*	5	2	95	1	1
	Vicia sativa	flag	*Bombus*	4	3	97	1	1
Lamiaceae	*Ajuga reptans*	gullet	*Bombus*	2	2	100	2	1
	Galeopsis tetrahit	gullet	long-tongued *Bombus* species	11	4	64	1	4
	Glechoma hederacea	gullet	*Bombus pascuorum, Apis mellifera*	15	4	82	2	3
	Lamium album	gullet	long-tongued *Bombus* species	12	4	73	2	4
	Lycopus europaeus	brush		11	4	54	4	6
	Mentha aquatica	brush		47	5	59	3	11
	Stachys palustris	gullet	*Bombus*	8	4	73	1	3

Plant family	Plant species	Flower type	known or assumed main pollinators	L_p[1]	N top 10 species	% top 10[2]	N 50%[3]	N 75%[4]
Lythraceae	*Lythrum salicaria*	brush	*Bombus pascuorum*	46	4	46	4	11
Onagraceae	*Chamerion angustifolium*	brush		43	7	77	4	6
	Epilobium hirsutum	tube		8	6	83	2	5
Primulaceae	*Lysimachia vulgaris*	dish	*Macropis europaea*	13	4	45	2	3
Ranunculaceae	*Ranunculus acris*	dish		64	3	62	3	9
	Ranunculus repens	dish		74	3	48	4	8
Rosaceae	*Filipendula ulmaria*	brush		24	5	84	2	4
	Potentilla erecta	dish	[apomictic]	36	4	6	1	1
	Potentilla reptans	dish	[apomictic]	5	0	0	1	1
	Rubus cf. fruticosus	dish	[apomictic]	70	6	50	3	11
Scrophulariaceae	*Linaria vulgaris*	gullet	long-tongued *Bombus* species	7	3	89	2	2
	Rhinanthus angustifolius	gullet	*Bombus pascuorum, B. hortorum*	6	2	82	1	2
	Veronica chamaedrys	dish	small Diptera	22	3	26	4	9
Valerianaceae	*Valeriana officinalis*	brush	*Eristalis horticola, E. intricarius, Helophilus*	58	6	45	5	12

[1] Lp (Linkage level)= the total number of insect species a plant species was visited by during the whole research period. [2] The proportion of top 10 insect species (table 2) of all visitors. [3] Number of insect species for a minimum of 50% of visitor frequencies (see text). [4] Number of insect species for 75% of visitor frequencies.

Table. 8.3. The number of insect species per functional group and in total contributing to 50% or 75% of the visitors of the 60 plant species (see text for explanations).

insect group	minimum 50% of visiting individuals	minimum 75% of visiting individuals	N insect species in remaining 25%	total
Flies	10	19	37	56
Syrphids	11	20	64	84
Beetles	1	6	40	46
Bugs	0	2	2	4
Butterflies	5	11	15	26
Moths	2	4	16	20
Ants	1	1	1	2
Wasps	1[1]	6[2]	70[3]	75[4]
Solitary bees	5	16	44	60
Bumblebees	2	7	7	14
Honeybee	1	1	0	1
other	0	0	8	8
Total	39	93	304	397

[1] Aculeate wasp. [2] Aculeate wasps: 3, parasite wasps: 2, Symphyta: 1. [3] Aculeate wasps: 29, parasite wasps: 26, Symphyta: 15. [4] Aculeate wasps: 32, parasite wasps: 28, Symphyta: 15

that visit a plant species (Olesen & Jordano 2002). Flower types and plant linkage levels are listed in table 8.2. Per flower type, the average of plant linkage level (fig. 8.1A), of the number of insect species contributing to 50% and to 75% of the visitors were calculated (fig. 8.1B), and the percentage of top-ten species of all visitors (Fig. 8.1C). Plant species with umbels had the highest mean L_p (108); twice as many as the flower type with the second highest mean L_p (umbel-head, 55), and more than 12 times as many visitor species as the group with the lowest mean L_p (flag, 7.5).

On average, less than four insect species are needed for a minimum of 50% of the visitors for each flower type (fig 8.1B). Plant species with umbels and umbel-heads had the highest number of insect species (3.8 and 3.7), and bell, tube gullet and flag lowest (between 1.0 and 2.0). For a minimum 75% of the visitors, the flower type flag still needs less than two species. For umbel, umbel-head and head this is more than nine on average. Bumblebees and *Rhingia campestris* are the main species groups for tube gullet and flag, for the other flower types these are (combinations of) flies, syrphids, solitary bees and bumblebees.

For the percentage of top-ten species the picture is completely opposite than linkage level and the number of species for 50 and 75%: umbel had the lowest (<30%), and flag the highest (almost 85%) contribution of top-ten species (fig. 8.1C).

The relation between the proportion of top-ten insect species and L_p was also analysed per plant species (linear regression, fig. 8.2). There was a weak negative relation between L_p and the proportion of top ten species (r^2= -0.15, $F_{1,58}$= 11.09, p< 0.005). Thus the

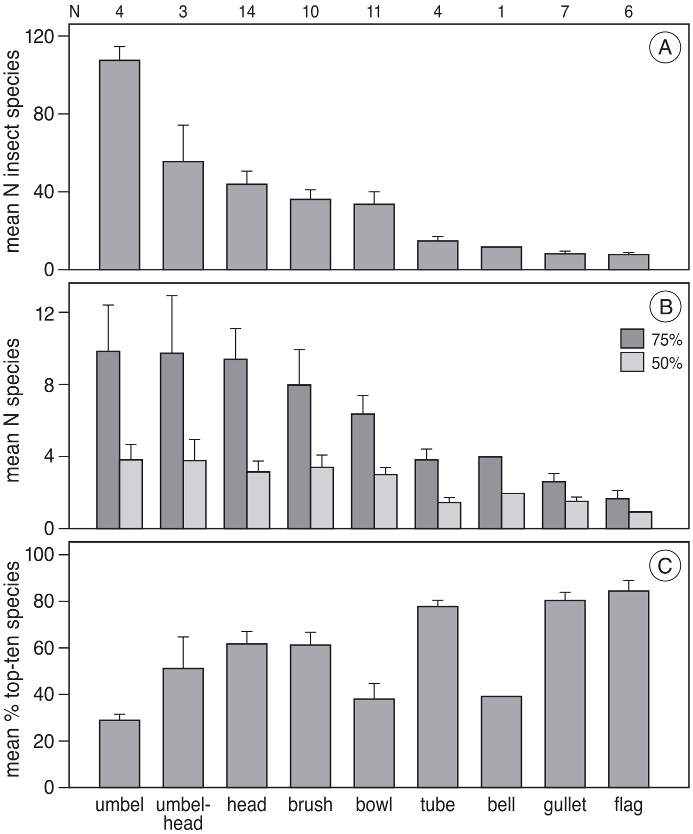


Fig. 8.1. (A) The mean number insect species contributing to 100% of visitors, (B) the mean number of insect species contributing to 50% or 75% of the visitors, and (C) the mean percentage of top-ten visitors (see text) of all visitors per flower type. The N-values are the number of plant species per flower type. The mean number insect species contributing to 100% of visitors is the same as linkage level (L_p). Error bars indicate SE.

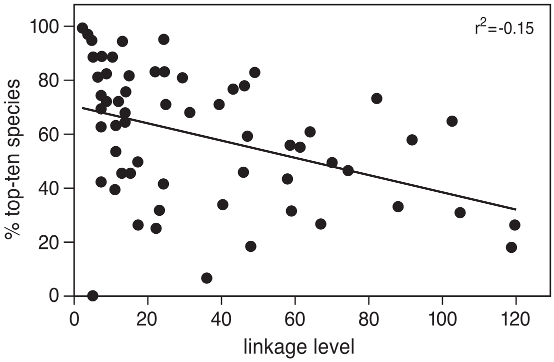


Fig. 8.2. Relation between Linkage level and the percentage of top-ten species of all visitors. Each data-point is a plant species (table 8.2).

lower the level of specialisation of a plant species, the larger is the "importance" of the top-insect species. *Hieracium pilosella* and *Jasione montana* were the plant species with the highest number of insect species observed for 50% of the visitors (8), for 75% these are *Cirsium palustre* (25), *J. montana* (22) and *H. pilosella* (20) (table 8.1).The lowest number of insect species for 50 and 75% was 1, for 19 and 7 plant species, respectively.

DISCUSSION

The most frequent and potentially most important visitor species, the "top ten", are all very abundant, common and generalist species. They occur during the whole or prolongued periods of the flowering season. The majority were Diptera (mainly syrphids), which is not surprising, as the flower-visiting fauna of northwest Europe is dominated by Diptera (Fægri & van der Pijl 1979; Ellis & Ellis-Adam 1993; Elberling & Olesen 1999; Memmott 1999). Three of the Diptera species (*Musca*, little brown fly and little black fly) are actually arrays of species that were not separated in the field for practical reasons (morphospecies, see Memmott 1999 and Dicks *et al*. 2002). This increases the importance of the other top-ten visitors at species level, particularly bumblebees are the most highly linked to many plant species (table 8.2). Memmott *et al*. (2004) also found bumblebees as the most important species in two North-American communities from the 1920s, but together with solitary bees. The top ten did not include any solitary bee, even though at least 45 species were observed. Solitary bees have steeply declined during the last century (Westrich 1996; Peeters & Reemer 2003) which may be an explanation. This is also true for butterflies (van Swaay & Warren 2001); butterflies occurred in low numbers in the research area of this study. Wasps and beetles are mainly restricted to a limited number of plant species (chapter 2, 3 & 4), and only one beetle species occurred in high numbers (table 8.2). A species that can occur in large number locally is the honeybee (*Apis mellifera*). In the Netherlands it is a domesticated species and there are 2.5 hives/ km^2 on average in the Netherlands (Brugge *et al*. 1998). Its local (but unpredictable) occurrence in high numbers, generalism and long flight period explain why *Apis mellifera* is in the top ten.

Three questions will be discussed now: Firstly, are the top-ten species enough for the pollination of this plant community? Secondly, if not, what other insect species are needed? And thirdly, what are the habitat requirements of the (minimal) pollinator diversity? Important aspects in this discussion are pollinator quality, degree of specialisation, spatio-temporal differences, plant population structure, and interactions between plants.

The top ten and other pollinator species

For the majority of plant species analysed (37 out of 60), the top ten insects contributed to more than 50% of the visitors, and for roughly one third even more than 75%. The latter were mainly specialised plant species with the flower types tube, gullet and flag, which were visited by the two bumblebees and *Rhingia campestris*. However, it was also clear that for a minimum of 50% of the visitors for all plant species, at least 39 insect species were needed. Identifying the actual pollinators among all flower visitors for all plant species in a community is extremely laborious (Memmott 1999; Olesen *et al*. 2002). As an indication, the assumed or known main pollinators of a number of plant species are listed in table 8.1. For many plant species, several top-ten species are among the main pollinators, particularly *Eristalis* and *Bombus* species. Most of the listed main pollinators are not among the top-ten species: they are important for only a limited number of plant species, occur only in low numbers, or have a short phenology.

Some plant species, the specialists, entirely depend on a limited number of pollinators. For two plant species, these are oligolectic solitary bees (chapter 6): *Lysimachia vulgaris* needs the bee *Macropis europaea*, and *Campanula rotundifolia* needs *Melitta haemorrhoidalis*. Bumblebees are the main pollinators for the specialised plant species, among them *Bombus pascuorum* and *B. terrestris*. But for plants with deeply hidden nectar in mainly zygomorph flowers, bumblebees with long probosces, like *B. hortorum*, are better pollinators. (Kwak 1979, 1988 & 1994a; Kwak *et al*. 1998). But *B. hortorum* had a lower frequency than the bumblebees in the top ten. For the plants the intermediate-tongued bumblebees (*B. pascuorum* and *B. terrestris* queens) may replace the long-tongued species, but only when nectar levels in the flower tubes are high enough so these bumblebees can reach them.

Pollinator quality

For some other top-ten species it was shown that their pollinator quality is low compared to other insects: *Rhingia campestris*, the most abundant visitor for *Phyteuma spicatum* and *Ajuga reptans* (chapter 2), is a poor pollinator compared to bumblebees (Kwak 1993). *Episyrphus balteatus* and the little brown and black Muscidae and Tachinidae flies were shown to be poor pollinators compared to several different insect species on a number of plant species, too (Velterop 2000; chapter 6).

Not only the pollinator quality, but also the relative abundance dictates pollinator importance. Locally mass-occurring species can be the most important pollinators by sheer numbers, even though their quality is poor compared to other species (Olsen 1997). In this study, *Bibio* species (table 8.1) are an example of such poor-quality pollinators (pers. obs.), that sometimes may still be important for Apiaceae species when *Bibio* visited the umbels in huge numbers (sometimes >50 individuals per umbel): incidentally up to 65% (*Anthriscus sylvestris*), 81% (*Angelica sylvestris*) or 94% (*Heracleum sphondylium*) of the visitors.

Spatio-temporal variation

Most plant species are generalists and therefore potentially have several possible pollinators. The composition and availability of those pollinators can vary strongly between locations (Bronstein 1995; Bingham & Orthner 1998; Kwak *et al*. 1998; Herrera 2000; Blionis & Vokou 2001; Fenster & Dudash 2001). For *Hypochaeris radicata* and *Leontodon autumnalis*, oligolectic solitary bees specialised on yellow Asteraceae (*Panurgus calcaratus, P. banksianus* and *Dasypoda hirtipes*) were the main pollinators at some sites, and syrphids (*Eristalis* species) at other sites in the study area (Hoffmann, unpub. data; van der Muren *et al*. 2003). Similar differences with other pollinator groups were also observed for *Anthriscus sylvestris, Jasione montana* and *Valeriana officinalis* (Kwak & Hoffmann, unpub.), and *Succisa pratensis* (chapters 5 & 6). Temporal and annual variation have similar consequences as local differences (Bronstein 1995): insect populations fluctuate strongly, and flower phenology of plants also varies between sites and years. The presence of several pollinator species may bridge those fluctuations for the generalist plant species. The spatio-temporal context of this study will have affected the composition of top-ten species in this study. The data set was from only two years and a restricted area; during a longer time span in a larger area the top-ten list may be different or even longer.

The structure of a plant population, i.e. flower density and patch size, have a strong effect on visitation and pollination (Kunin 1993; Ågren 1996; Kwak *et al*. 1998; Bosch & Waser 1999; Luijten *et al*. 2000; Mustajärvi *et al*. 2001; chapter 7). For example, short-tongued bumblebees are more common in large and dense patches, whereas intermediate- and long-tongued bumblebees are attracted more by lower density patches (Sowig 1989; Kwak *et al*. 1998). As both high and low density patches can occur within one plant population, the presence of several bumblebee species may be necessary. Similarly, honeybees and bumblebees preferred larger patches and solitary bees smaller patches in one study (Sih & Baltus 1987). Behavioural differences between insect species can also be important in other spatial contexts. For example, butterflies that are generally relatively poor-quality pollinators in Europe (Jennersten 1984), may contribute to gene flow as they fly relatively large distances between flowers (Velterop 2000).

Habitat requirements of the pollinator community

It is very likely that the top ten of flower visitors is not enough for a successful, long-term guaranteed pollination of all of the 60 plant species in the community presented. Some of the most important visitors and pollinators are the 30 species listed in table 8.5, based on the top-ten species and most of the insect species from table 8.1. It is not intended to be complete, but to give an impression of the habitat requirements of the pollinator fauna. The species belong to various taxonomical groups and have very different life histories and habitat requirements. Their phenologies vary between the species and within genera. Some are only present early (e.g. *Empis* species, *Andrena haemorrhoa* and *Bombus pratorum*), or in summer (particularly bees), and some species are present during the whole year. Particularly the top-ten species have a long flight season.

The Diptera (flies and syrphids) are non-central place foragers, i.e. they do not take care of the offspring and can in theory forage anywhere. They have various food sources: some species are omnivorous (e.g. *Musca* spec.) or partially carnivorous (e.g. *Empis* spec.). For them nectar and pollen are only part (though sometimes substantial) of their diet. Most syrphids eat pollen and also nectar, and depend on flowers during their adult stage. The larvae of all of these Diptera species have entirely different food sources than the adults (from rotting materials to aphids), in often totally different habitats than the foraging sites of the adults (terrestrial, aquatic, dung, etc.). Therefore habitat heterogeneity is an important aspect for these species in fulfilling their life cycle (Verberk *et al*. 2002; Chust *et al*. 2003).

All bees are central-place foragers (Bronstein 1995), i.e. animals that do some kind of care for their brood and have to take food to a nest, so they cannot forage too far away from a fixed site (at least the females). Most bees are solitary (table 8.5) and some are social (*Lasioglossum calceatum*, the bumblebees and the honeybee). Most of the listed species have terrestrial or belowground nests. Several bees need dry soil or even open, dry and sandy soil. Many bee species (not listed) make their nests in plant material or wood (Westrich 1990). The ground-nesting bumblebees and wasps often prefer abandoned vole or mole holes. For example, population dynamics of bumblebees and wasps and the spring-migration behaviour of their queens were related to population cycles of voles in Finland and Estonia (Vepsäläinen & Savolainen 2000). Solitary bees have short foraging distances, up to several hundreds of metres from their nest (Westrich 1996; Calabuig 2000), whereas for bumblebees and honeybees this can be up to 10 kilometres, but usually not more than two kilometres (Saville *et al*. 1997; Osborne *et al*. 1999; Dramstad *et al*. 2003). But also between bumblebee species foraging distances vary considerably (Walther-Hellwig & Frankl 2000). Thus for bees, both food sources and nesting sites need to be within their flight (foraging) range. Like the Diptera, they also depend on habitat heterogeneity, or "partial habitats" within one ecosystem (Westrich 1996).

Flower community requirements of the pollinator community

Insects have differing preferences for and abilities to visit certain flowers (Fægri & van der Pijl 1979). The flower composition of a vegetation therefore affects the presence or breeding success of those insects. All flower types have at least one insect species for which it was a major part in the diet of the insects in table 8.4. The type head was most often found (23x), followed by brush (17x), umbel (15x) and bowl (12x). These flower types are easily accessible also for short-tongued insects and therefore needed in a vegetation. Tube (4x), gullet (6x) and flag (5x) flowers are only accessible for intermediate to long-tongued species, or species with good learning capabilities. Long-tongued bumblebees (*B. hortorum, B. pascuorum*) prefer these deep flowers, whereas short-tongued bumblebees (*B. pratorum, B. lapidarius*) prefer more open flower types (Rasmont 1988; Fussell & Corbet 1992; Osborne & Corbet 1994; Mänd *et al*. 2002). For a long-tongued bumblebee drinking nectar from a shallow flower is "like drinking a glass of lemonade with a garden hose for a person" (M.M. Kwak, pers. comm.). The flower types head and brush supply pollen and/ or nectar for many flower visitors with various proboscis lengths, and are therefore crucial in a vegetation. Several of the apomictic plants (e.g. *Hieracium*) therefore are important food sources, even though they may hardly need pollination.

Flower visitors need food supply during there whole life cycle. For early species there should be enough spring flowers, and species that hibernate as adults (some *Eristalis* species and bumblebee queens) need late-flowering plant species for building up reserves (Bronstein 1995). Bumblebees and other species with long flight periods (at least the colonies) need continuous food supply from the start of the season; food supply in spring will affect colony growth in the rest of the season (Heinrich 1979; Fussell & Corbet 1992; Goulson *et al*. 2001). Late flowering species in the area are e.g. *Leontodon autumnalis* (very common) and *Succisa pratensis* (a rare and endangered species). Long-tongued

Table. 8.4. Characteristics and habitat requirements of some of the most important visitor and pollinator species for the 60 plant species in this study (table 1). Literature resources: (van der Goot 1990; Achterkamp *et al*. 1998; Chinery 1998; Peeters *et al*. 1999; Speight *et al*. 2001). The major flower types are based on visitation patterns in this study.

species	insect group	phenology	nest	food	major flower types
Musca spec.	fly	whole season	-	omnivorous; larvae: rotting materials	umbel, head, brush, bowl
little brown fly	fly	whole season?	-	flower products?	umbel, head, bowl, umbel-head
little black fly	fly	whole season?	-	flower products?	umbel, head, brush, bowl
Scathophaga stercoraria	fly	IV-IX	-	carnivorous, nectar; larvae: dung	umbel, head
Empis diagramma	fly	IV-V, (VI)	-	carnivorous, nectar; larvae: carnivorous?	umbel
E. livida	fly	IV-VII	-	carnivorous, nectar; larvae: carnivorous?	head, umbel, bowl, brush
E. tessellata	fly	IV-V, (VI)	-	carnivorous, nectar; larvae: carnivorous?	umbel
Bibio marci	fly	IV-V	-	nectar; larvae: ?	umbel
Episyrphus balteatus	syrphid	(III-VI), VII-X, (XI)	-	pollen; larvae: aphids	all, incl. wind-pollinated plants
Eristalis tenax	syrphid	(III-VI), VII-X, (XI)	-	pollen, nectar. larvae: aquatic, dung pits, bacterivorous	head, brush, umbel
E. arbustorum	syrphid	IV- IX	-	pollen, nectar. larvae: aquatic, dung pits, bacterivorous	umbel, head, brush
E. horticola	syrphid	V-VI, (VII), VII-IX	-	pollen, nectar. larvae: aquatic	umbel, brush
E. intricarius	syrphid	IV-IX	-	pollen, nectar; larvae: aquatic	brush, head
Helophilus pendulus	syrphid	IV-XI, (X)	-	pollen, nectar; larvae: humid rotting materials, dung	brush, head, umbel, bowl
Rhingia campestris	syrphid	IV-V, (VI-VII), VII-IX	-	nectar, pollen; larvae: aquatic, moist cattle dung	brush, gullet, head, tube
Andrena haemorrhoa	solitary bee	(III), IV-V, (VI)	diverse nesting sites	pollen and nectar	head, umbel, bowl
Lasioglossum leucozonium	solitary bee	V-IX, (X)	belowground	pollen and nectar	head
Macropis europaea	solitary bee	(VI), VII-VIII	belowground, hidden in vegetation	pollen and oil of *Lysimachia*; nectar of other plants	bowl; brush, head
Melitta haemorrhoidalis	solitary bee	(VI), VII-VIII, (IX)	belowground in dry soil	pollen and nectar of Campanulaceae	bell
Dasypoda hirtipes	solitary bee	VI-VIII	belowground in dry soil	pollen of Asteraceae, nectar diverse	head, umbel-head
Panurgus calcaratus	solitary bee	VII-VIII	belowground in open dry soil	pollen of yellow Asteraceae, nectar diverse	head
P. banksianus	solitary bee	(V), VI-VII, (VIII)	belowground in open dry soil	pollen of yellow Asteraceae, nectar diverse	head
Lasioglossum calceatum	social bee	IV-IX, (X)	belowground	pollen and nectar	head, bowl
Bombus pascuorum	bumblebee	III-X	mostly belowground	pollen and nectar	tube, gullet, brush, head, flag
B. terrestris	bumblebee	II-X	belowground	pollen and nectar	all, except bell
B. hortorum	bumblebee	III-IX	in or on soil	pollen and nectar	gullet, flag, tube
B. pratorum	bumblebee	(II), III-VI, (VIII-IX)	in or on soil	pollen and nectar	brush, bowl, tube
B. lapidarius	bumblebee	IV-VIII, (IX-X)	under stones or tree roots	pollen and nectar	head, flag, brush
B. jonellus	bumblebee	III-VI, (VII-IX)	in or on soil	pollen and nectar	gullet, head, brush, bowl
Apis mellifera	honeybee	IV-XI	domesticated	pollen and nectar	brush, head, umbel-head, bowl

species need gullet or tube flowers early in the season (Osborne & Corbet 1994; Kwak *et al*. 1998), for example *Glechoma hederacea* and *Lamium* species. Also mass-flowering trees and bushes (e.g. *Prunus* and *Crataegus* species) are major food sources for early species (Osborne & Corbet 1994; Westrich 1996). Mowing is a temporary removal of flowers and disrupts food supply if it is on a large scale. Some sites in the research area were mown repeatedly, and in the intensively used agricultural parts no trees or bushes are left that may provide early food sources or alternatives for mown sites (chapter 3).The bees depend on flower products during their whole lifecycle. Generalist bee species are called polylectic. Many solitary bees are oligolectic, i.e. specialised on pollen of one or several related plant species. They need their particular host plants for offspring production. However, oligolectic bees are generalist nectar foragers, and may get nectar from any available plant species (Bronstein 1995; Skov 2000). For Macropis europaea the presence of other plant species than its pollen and oil supplier Lysimachia is even a must, as this plant does not produce any nectar.

Conclusions

The answer to the first question (Are most plant species in a community visited by a small set of insect species?) is yes: Only ten insect species (2.5% of the species observed) constituted more than 50% of the visitors of two thirds of the 60 analysed plant species in the studied community. Particularly the specialist plant species with generalist visitors have a high proportion of top-ten visitors, mainly bumblebees.

However, the answer to the second question (Are those insect species responsible for the *pollination* of the majority of the plant species?) is no. Firstly, some of the dominant visitors (the top-ten species) are not or low-quality pollinators. Secondly, many more pollinator species are needed for the pollination of the plant community for reasons involving pollination quality, degree of specialisation, spatio-temporal differences, plant population structure, and interactions between plants. Generalist plant species may have various alternative pollinator species, but these species need to be present. Specialist plants need bumblebees and solitary bees for their pollination, including low-abundance species. Therefore it is unlikely that the dominant insects are keystone species in the system, although the importance of some of them is undoubted.

A good deal of habitat heterogeneity, continuous food supply throughout the flowering season and different flower types are needed for sustaining the pollinator fauna. Thus for conservation of plants "extended care" is needed: it goes further than the plant's growing needs and sites, and should also include the habitat of their pollinators (Tepedino *et al*. 1997).

9

General discussion

Frank Hoffmann

INTRODUCTION

Ecological levels

The aim of this thesis is to investigate whether a high biodiversity is important for the pollination of insect-pollinated wild plant species in a context of agricultural land use. The answer to the question about the importance of biodiversity for pollination will differ between ecological levels: at plant community level, a high pollinator diversity can be important for the pollination of all plant species (chapters 2-4 and 8), while an individual plant species will often only need its particular pollinators (chapters 5-7). Patterns observed at community level are not necessarily observed at species level: in chapter 5, plant species richness was not correlated with plant linkage level (the number of visiting insect species) of some selected plant species, while in chapter 4 the mean linkage levels of all plant species combined were correlated with plant species richness.

In chapter 1, I mentioned three types of hypotheses about the relation between biodiversity and ecosystem functioning, that can also be applied to the relation between biodiversity and pollination. Firstly, the redundancy type (all species in an ecosystem or community are equal), secondly, the keystone-species type (a pollination community is stabilised by several keystone species or "interaction nodes" in a plant-pollinator network), and thirdly, the context-dependence type (the effect of biodiversity depends on the context). Although these hypotheses are meant for ecosystems or communities, the ideas can also be applied to individual species. In this chapter I will discuss which type of hypotheses can be applied to the relation between biodiversity and pollination at community and plant species level.

Biodiversity includes the diversity of plants and insects, that have different effects on pollination (see chapters 5, 6 and 8), but that are also highly related to each other in various ways (chapters 3 and 4). Diversity can be regarded as species diversity, but also functional diversity (the flower types and insect groups introduced in chapter 2). Both of them were used in several chapters. The answer to the main question and the type of hypothesis thus also depends on whether biodiversity means the total plant and insect diversity, plant diversity or insect diversity .

Topics for discussion

Landscape or land use (the matrix of habitats with the various types of land use), plant and insect community (species richness, abundances of flowers and insect individuals, and functional and species compositions in the road verges), individual behaviour, visitation of flowers, pollination and seed set are the core aspects and processes that are thought to be important concerning the role of biodiversity for pollination (chapter 1, fig. 1.3). Table 9.1 shows in which chapters these aspects were studied. One important aspect, the individual behaviour of insects, got relatively little attention and was only directly measured in chapter 7. Therefore I will pay special attention to the individual behaviour of insects in relation to biodiversity at the beginning of the discussion.

The importance of biodiversity will be discussed according to ecological level (community or species). Furthermore, I will discuss spatial and temporal aspects, such as habitat fragmentation, landscape history, long-term processes.

Table. 9.1. Overview of subjects per chapter. Indicated are the effects of landscape or land use, site management, plant community and insect community on plant community, insect community, individual behaviour, visitation of flowers, pollination and seed set. The numbers in the table are the chapters in this thesis; in bold are the main chapters for the subjects. The interactions between the different aspects are shown in chapter 1 (fig. 1.4). Chapter 2 is not indicated in the table, because it is a description of the functional diversity of flowering plants and the flower visiting insects.

Effect studied on	Landscape/ land use	Plant community	Insect community
Plant community	**3**, 8		**8**
Insect community	**3**, 8	**3**, **4**, 8	
Individual behaviour		7	
Visitation	**6**, **7**	**4**, **5**, 6, **7**	**4**, **6**, **7**, **8**
Pollination	**6**, **7**	**5**, 6, **7**	5, **6**, **7**, 8
Seed set	**6**	5, 6, 7	5, **6**

Analysing the effect of the management (mowing) of road verges and ditch banks on pollination was not the goal of this thesis, but due to its frequent occurrence it cannot be ignored. Data about the effects of mowing on flowering and flower visitation available from this study will be shown in box 9.1. Management and nature conservation in general will be discussed at the end of this chapter.

Pollination involves two parties (insects and plants) and can and should be regarded from the point view of both parties (Waser 2001). But since the function of pollination is the reproduction of plants, this discussion will be from that point of view, and I will switch to the insects point of view when required.

INSECTS: BEHAVIOUR OF SPECIES AND INDIVIDUALS

Frequencies and pollinator qualities

As this thesis is about pollination of entomophilous plants, apart from plants, insects are also of central interest: their behaviour determines the reproductive success of the plants (Goulson 1999). The insect's behaviour can be studied at species or individual level, but the choices made by the individuals determine pollination (Kwak *et al*. 1998). The frequencies and behaviour of insect species gained most of the attention in this thesis, while the behaviour of individuals was only studied in chapter 7. In other chapters the net effect of the behaviour of the individuals combined was measured as pollination (chapters 5, 6 & 7) or seed set (chapter 6). In chapter 8 it was stressed that insect species that seem to be "important" as pollinators due to their frequencies (chapters 2-4), may appear to be less important or even negligible when the number of visits or other pollination qualities are considered. Features of pollinator quality of individual insects are among others visitation speed, the number of deposited pollen grains on stigmas per visit, and flower constancy (Fægri & van der Pijl 1979; Dafni 1992; Kwak *et al*. 1998; Goulson 1999; Thomson & Goodell 2001; Waser 2001; Slaa & Biesmeijer 2003).

Visitation speed

Many of the data in this study are from transect observations (e.g. chapters 2-5), i.e. frequencies of insect species. With transects many data could be collected in a short period of time, but these data can also over- or underestimate the pollination role of some insect species. Insect species that are relatively infrequent can turn out to be the most important pollinators (Kwak 1980; Kwak *et al*. 1998; Mayfield *et al*. 2001). Examples of the differences between frequencies of individuals and of the number of visits are given in fig. 9.1. Visitation speed, the number of flowers visited per individual per unit time, can explain why there are such large differences between insect taxa. Of some insect species in the research area of this study visitation speeds were measured (table 9.2). The visitation speeds of the insect species appear to vary considerably per plant species. Particularly some oligolectic solitary bees (e.g. *Dasypoda hirtipes* on yellow Asteraceae, *Melitta haemorrhoidalis* on *Campanula rotundifolia*, and bumblebees on *Succisa pratensis* and *Glechoma hederaceae*) can be much faster than other species (table 9.2). Also within Diptera there are considerable differences, as illustrated by *Anthriscus sylvestris* and *Succisa pratensis* (table 9.2). From the plant's point of view, the positive effect of a quick insect is that the insect visits many flowers within a short period of time, spreading and depositing pollen on many flowers. Thus while frequency data can give a good insight into general patterns of visitation, the researcher should be aware that frequencies do not direcly show which insects are actually important pollinators.

Pollen deposition

High speed of an insect can also have a negative side: the deposition of pollen on stigmas requires some time, and when an insect moves away too quickly, the chance for deposition may decrease. The amount of deposited pollen per insect is insect species-specific and depends partially on how well an insect body fits the morphology of a plant (Fægri & van der Pijl 1979). Some of the fastest insect species in table 9.2 can also deposit the highest amounts of pollen: bumblebees deposit much more pollen per visit than the small syrphid *Rhingia campestris* on *Phyteuma spicatum* subsp. *nigrum* (Kwak 1993) or bumblebees and the honeybee on *Glechoma hederaceae* (Kwak, unpub.). No data are available for the oligolectic solitary bees visiting the yellow Asteraceae, but a related species to *Dasypoda hirtipes*, *D. argentata*, was not only the fastest, but also deposited the most pollen grains on *Scabiosa columbaria* in France (Velterop 2000). Large syrphids can deposit slightly less to similar amounts of pollen per visit compared to bumblebees on e.g. *Succisa pratensis* (Kwak 1993) and *Scabiosa columbaria* (Kwak 1993; Velterop 2000).

Flower constancy

Flower constancy is an aspect of individual behaviour that is much related to plant diversity. Flower constancy is the tendency of a flower-visiting animal to restrict its visits to flowers of a single plant species, ignoring rewarding flowers of other species present in a vegetation (Fægri & van der Pijl 1979; Waser 1986; Slaa & Biesmeijer 2003). To understand this phenomenon one has to switch to the point of view of the foraging insect. Insects visit flowers to obtain food, mostly nectar and pollen (Harder *et al*. 2001). Nectar is an easily

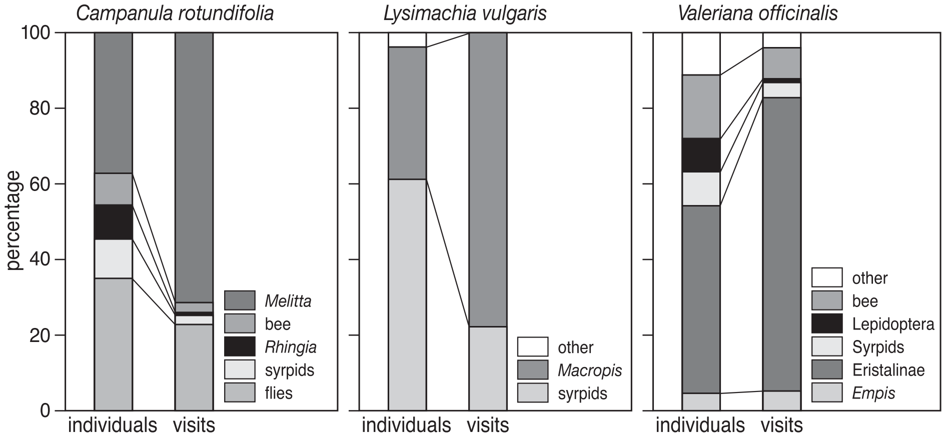


Fig. 9.1. Comparison of frequencies of individuals (measured in transect walks) and frequencies of visits (measured in continuous 10-minute observations) of insect taxa for three plant species: *Campanula rotundifolia*, *Lysimachia vulgaris* and *Valeriana officinalis*.

Table. 9.2. Visitation speeds of a number of insect species at various sites and dates in the study area. Individual insects were followed during foraging bouts until they were lost out of sight. The number of flower units visited was scored per individual. Part of the data were collected by Maaike de Vlas, Henk Hunneman and Diana Prins, for which I am very grateful.

Plant species (flower unit)	Insect species	insect type	N	N flower units/ min ±SE
Anthriscus sylvestris (umbellule)	*Tachina fabricia*	fly	5	13.1 ±2.0
	Eristalis tenax	syrphid	12	10.2 ±1.4
	Eristalis arbustorum	syrphid	8	9.6 ±0.9
	Empis tessellata	fly	4	6.5 ±2.1
	Musca species	fly	4	4.1 ±0.6
Yellow Asteraceae (head)[1]	*Dasypoda hirtipes*	solitary bee	14	14.9 ±1.8
Yellow Asteraceae (head)[2]	*Panurgus calcaratus*	solitary bee	10	14.0 ±1.2
Yellow Asteraceae (head)[1]	*Eristalis tenax*	syrphid	3	7.5 ±2.1
Succisa pratensis (head)	*Bombus pascuorum*[3]	bumblebee	7	4.8 ±0.8
	Eristalis horticola	syrphid	15	2.9 ±0.4
	Helophilus trivittatus	syrphid	25	2.6 ±0.4
	Helophilus pendulus	syrphid	19	1.6 ±0.2
Glechoma hederacea (flower)	*Bombus pascuorum*	bumblebee	6	11.8 ±2.0
	Apis mellifera	honeybee	14	9.4 ±1.5
	Rhingia campestris	syrphid	14	9.2 ±4.1
Campanula rotundifolia (flower)	*Melitta haemorrhoidalis*	solitary bee	12	9.2 ±2.2
	Syphona species	fly	5	<<1[4]

[1] *Hieracium umbellatum* and *Leontodon autumnalis*. [2] *Hypochaeris radicata* [3] From Kwak (1993). [4] These small flies stayed very long in flowers of *Campanula*.

digestible energy source, while pollen is rich in proteins and fats, and an important protein source for bees and many syrphids (Harder *et al*. 2001). Like all animals, insects must make economic decisions while foraging: choices between flower species, choices for foraging site and patches, and choices between individual flowers (Goulson 1999). The ability to make choices between plant species can be determined by an insect's ethological abilities (e.g. limitations in nervous system) and physical abilities (morphology of a species to reach certain flowers). These are called intrinsic causes of constancy (Waser 1986; Goulson 1999). However, some level of flower constancy is a general phenomenon among different insect taxa, and not only for bees (Goulson 1999; Weiss 2001). Examples of flower constancy are observed for some Lepidoptera (Goulson *et al*. 1997a & b) and three species of syrphid flies, i.e. *Eristalis tenax*, *Syrphus ribesii* and *Episyrphus balteatus* (Haslett 1989; Goulson & Wright 1997; Sutherland *et al*. 1999). In chapter 7 it was shown that there are differences in flower constancy between syrphid species: *Helophilus pendulus* was less flower constant than *H. trivittatus* and *Eristalis horticola*. Obligate, often inherited food specialisation can be another intrinsic cause for flower constancy, or rather flower preference, because constancy implies a temporal specialisation (Waser 1986). Oligolectic and monolectic solitary bees are a good example of such specialists. Among bumblebees it is known that the long-tongued species (*Bombus hortorum*, *B. pascuorum*) have a preference for deep flowers, whereas short-tongued bumblebees (*B. pratorum, B. lapidarius*) prefer more open flower types (Rasmont 1988; Fussell & Corbet 1992; Osborne & Corbet 1994; Mänd *et al*. 2002).

Extrinsic causes for flower constancy are flower abundance, density and composition of flowers and the rewards therein (Waser 1986; Goulson 1999). A higher flower constancy can be expected to be more efficient (optimal) as it can reduce the costs of searching and handling time of different flowers, but it is unclear whether these benefits really outweigh the costs of increased travelling time under flower-constant foraging and are thus sub-optimal (Slaa & Biesmeijer 2003). The type or quality of plant species will determine much of the behaviour. For example, pollinators are more flower-constant when flower types are more distinct, as they are easier to distinguish for insects (Waser 1986; Chittka *et al*. 1997; Hill *et al*. 2001; Slaa & Biesmeijer 2003). This was observed for syrphid flies (*Eristalis* and *Helophilus* species) in an array experiment with *Succisa pratensis* heads in combination with other flower species (Kwak & Hoffmann, unpub.). Similarly, interactions between plants, mediated through insect behaviour, are more likely to occur between plant species that are more similar (chapter 5). However, inconstancy does not need to imply inability to distinguish flowers, as sometimes there may be no energetic difference between similar flowers, and therefore no need for the forager to select (Thomson 1981). Other causes can be differences in nectar composition, such as nitrogen and phosphorus contents. Furthermore, even during flower-constant foraging, bumblebees take samples from other plant species to keep track of changing rewards in time, this is known as "minoring" (Heinrich 1979b).

Effects of biodiversity: linkage level and flower constancy

The number of species in a network a particular species interacts with, like the number of plant species an insect visits, is called linkage level (Olesen *et al*. 2002; Olesen & Jordano 2002; Dupont *et al*. 2003). In different communities, regardless of climate, the majority of insect species visits only two to five plant species on average (Olesen & Jordano 2002). A similar number was observed in this study (chapter 4): the mean insect linkage level was 1.29 for all insects, and the maximum number of plant species visited by an insect species was seven. However, the linkage level of the insect species varied with plant species richness; when there were more flower species at a site, an average insect species was observed on more flower species (chapter 4). Even though there were differences between different taxonomic groups, this phenomenon was observed for all insects. Thus at species level the insects tend to visit more plant species, but the question is whether this is also the case with individuals. Do individuals within a species visit as many plant species as the insect species as a whole, or do individuals within an insect species visit only one plant species each, but different ones? In this thesis flower constancy was only directly measured for syrphid flies on *Succisa pratensis* (chapter 7). Syrphid species differed slightly in flower constancy, but this was probably also related to the population size of *S. pratensis* and not only flower diversity. The effect of population size was also reflected in the stigmatic load, which is a combined result of all individuals that visited a flower. In larger populations the proportion of heterospecific pollen was lower than in smaller populations. As said above, (relative) abundances of different species can have a strong effect on constancy. When flowers of all species combined are scarce, foragers will abandon temporal specialisation and forage on several plant species. This has been observed for honeybees, bumblebees and syrphid flies (Kunin 1993; Chittka *et al*. 1997; Goulson 1999; Waser 2001).

Thomson (1981) observed that the constancy of bumblebees decreases with increasing floral diversity. This is in line with what was observed for linkage levels. Bumblebees can be flower-constant, but still individuals often carry mixed pollen loads in the corbiculae (Kwak & Jennersten 1986; Kwak & Bergman 1996). Also syrphid flies carry mixed body loads (Velterop 2000). The body pollen loads of the syrphids analysed in chapter 7 contained large amounts of heterospecific pollen of plant species from further away, even in large *S. pratensis* populations. This means that they foraged on larger spatial scales than only the size of the *S. pratensis* population. Probably insects are flower-constant within a patch, but might be less constant on larger scales. Bumblebees are flower-constant as long as flowers are rewarding and close enough, but they switch to other plants when flowers have low rewards, or are not encountered closely enough (Chittka *et al*. 1997; Kwak & Vervoort 2000). But sometimes learned preference may be stronger than flower-frequency effects on constancy and flower choice (Smithson 2001).

The effect of plant species richness on linkage levels and constancy differs between insect groups: while flies, syrphids and bumblebees were observed on more plant species as there were more available, this was not the case for solitary bees (chapter 4). Within bees, honeybees are often more flower-constant than bumblebees (Thomson 1981; Velterop 2000), and within syrphid flies, *Eristalis horticola* and *Helophilus trivittatus* are more constant than *H. pendulus* (chapter 7). Bees are more "truly" or actively specialised than other flower visitors; they have only flower products such as nectar and pollen in their diet (Ellis & Ellis-Adam 1993). Bumblebees and honeybees are social insects with a long flight period (the whole season) and therefore can forage on many plant species, whereas for solitary bees with flight periods of a few weeks (Westrich 1990) this is much less the case (Bronstein 1995). Whether solitary bees are more constant than other insects is unknown, but probably unlikely, as there are many generalist species. Solitary bees have short foraging distances, up to several hundreds of metres from their nest (Westrich 1996; Calabuig 2000). When flowers are scarce and distributed over several plant species the bees have to forage on more than one plant species. But oligolectic bees are expected and sometimes observed to be the most constant due to their specialisation and preference (Bronstein 1995; Kwak *et al*. 1998; Velterop 2000). However, their specialisation is only based on pollen; the bees may get nectar from any available plant species, and therefore may receive heterospecific pollen grains (Bronstein 1995; Skov 2000; Waser 2001). Furthermore, flower preferences, including those of many oligolectic bees, are mostly not restricted to one plant species, but either include several taxonomically related species, or species with morphological and chemical similarities. For example, I have observed that oligolectic bees foraging on yellow Asteraceae, like *Dasypoda hirtipes* or *Panurgus calcaratus* visit the nearest "yellow flower head" they encounter, irrespective of whether this is for example *Hypochaeris radicata, Hieracium laevigatum* or *Leontodon autumnale*. Experi-ments with mixtures of these related plant species may reveal whether this impression is true. In such a small-scale array experiment the syrphid *Eristalis tenax* distinguished only some species pairs of yellow Asteraceae, which may be related to UV-reflection (visible for many insects) of some the species (van der Muren *et al*. 2003). In another experiment (unpub. data), *Succisa pratensis* and *Scabiosa columbaria* were combined with five arrays with other plant species that varied in resemblance with the target species (from very similar and closely related species to very different species). Large syrphids (*Eristalis* and *Helophilus* species) were more flower-constant on *S. pratensis* and *S. columbaria* as the flowers were less similar.

The diversity of insects may also affect individual behaviour through interactions between insects species. For example, scent can be used by bumblebees to reject flowers that were visited by other bumblebee species (Goulson 1999). Also depletion of resources (competition for food) plays a role. One example is the competition between domesticated honeybees and native pollinators (Brugge *et al*. 1998; Paton 2000; Goulson 2003). However, from the results of this thesis nothing can be said about interactions between insect species.

Individual insects: summary

The behaviour of species does not necessarily give a clue about the individual behaviour. Scoring only frequencies can be misleading as an estimate of pollination quality, and visitation speed can provide more information. Similarly, linkage levels do not tell how flower-constant an individual is. Frequencies are observations of single moments in time, while individual behaviour shows what happens during a longer time period. The individual behaviour differs between species and even within species between individuals. Extrinsic and intrinsic factors determine the individual behaviour. Even though optimal foraging may favour insects energetically, from the plant's point of view this does not need to be optimal. Firstly, insects may forage on many different species. Secondly, plants are confronted with both optimal and sub-optimal foragers; individuals of the same species may show differences in behaviour even while facing the same problem, probably due to differences in experience (Thomson & Chittka 2001; Waddington 2001). More data are needed about the individual pollination behaviour in relation to biodiversity of various flower-visiting taxa.

POLLINATION AND BIODIVERSITY: INDIVIDUAL PLANT SPECIES

Effects of plant diversity

The effect of plant diversity on pollination is plant species specific, and depends on the type of neighbouring species rather than total plant diversity (chapter 5). The balance between facilitation and competition depends on plant population size and flower density of both the target and other plant species in the community, and on plant community species composition. In a neighbourhood with a high plant diversity visitation rate on *Scabiosa columbaria* was higher than in a low diversity neighbourhood, but there was a negative effect on pollination (chapter 5). Above it has been mentioned that interactions between plant species, mediated through individual and species behaviour of the flower visitors, are more likely between plant species with floral similarities, or that share part of their pollinators. These are short-term processes that will take place within one or several days. They depend on the flowering phenology and particularly the duration of the viability of pollen, which is usually only a few hours or exceptionally some days (Primack 1985; Dafni 1992; Murren 2002).

Long-term processes (longer than the flowering period of a plant) are more difficult to measure, and have not been investigated in this thesis. These processes involve indirect dependence of a plant species on other plant species in time. Flower visitors need food supply during their whole life cycle. For example, bumblebees and other insects with long phenologies need continuous food supply from the start of the season; food supply in spring will affect colony growth in the rest of the season (Heinrich 1979; Waser & Real 1979; Fussell & Corbet 1992; Goulson *et al*. 2001). Similarly, insects hibernating as adults need flowers late in the season for building up reserves (Bronstein 1995). Thus plants can indirectly depend on plant species flowering in other periods of the season than they do themselves when they depend on long-lived or hibernating insects. Thus short-term effects (during the time a plant is flowering) of plant diversity can be neutral, positive or negative through direct interactions, but long-term indirect effects are likely to be beneficial. More data are needed to highlight these processes.

Effects of insect diversity

There are only few studies that show the negative effect of insect species decline for single plant species (Kwak *et al*. 1998). Examples are *Dianthus deltoides*, where a decrease of insect species richness led to a decrease in seed set (Jennersten 1988), or *Primula sieboldii*, where loss of pollinators resulted in a breakdown of the breeding system (Washitani 1996). The data in this thesis showed some potential dangers of pollinator species loss. A single plant species does not depend on insect diversity as a whole, but only needs its particular pollinators (chapter 6). Plant species that have several alternative pollinators usually do not need the whole set of species at a certain place, but only one or a few. On a larger spatial scale, however, insect diversity may be important due to variation in insect assemblages between flower patches and populations (chapters 6 & 7; Bronstein 1995; Bingham & Orthner 1998; Kwak *et al*. 1998; Herrera 2000; Blionis & Vokou 2001; Fenster & Dudash 2001). Generalist plant species can have several pollinators, but not all insect species are always present due to temporal or annual fluctuations of insects (Schoenly & Cohen 1991; Ottenheim 2000; Verberk *et al*. 2002). Thus on longer time scales the presence of several pollinator species may overcome these fluctuations (Bronstein 1995; Fishbein & Venable 1996; Fenster & Dudash 2001; Mayfield *et al*. 2001; Kandori 2002). For example, *Succisa pratensis* received hardly any bumblebee visits in 2002, and many visits from *Bombus pascuorum* in 2003, but in both years it was visited by *Eristalinae* syrphids, that are also relatively good pollinators (chapter 7).

Life history and pollination biology

Life-history features, such as life span and clonality of plant species also affect susceptibility to (temporal) pollinator loss. Annuals need pollination every year, and the soil seed bank will need regular input from seed rain (Bakker *et al*. 1996; Thompson *et al*. 1997), particularly when seeds have a short longevity (Bekker & Kwak 2005; Kwak & Bekker 2005). The latter is also true for longer-lived monocarp species like biennials. Thus for short-lived species pollination deficiency can have direct consequences for the plant populations. Perennials may escape pollination deficiency or effects are hidden through long life spans, or some species through strong vegetative reproduction and clonal growth (Kearns *et al*. 1998; Kwak *et al*. 1998; Donaldson *et al*. 2002).

Facultative selfing may prevent plant species from going extinct when pollinators are limited or absent (Kearns *et al*. 1998; Kwak *et al*. 1998; Spira 2001). However, facultative selfing is predicted to be no long-term solution for permanent pollinator loss (Moldenke 1975). Indeed, in chapter 6 it was illustrated that seed set of the plant species with potential self-pollination capacity was considerably reduced without insect visitation. Similar results of reduced seed set under autonomous selfing were found for *Collinsia verna* (Kalisz & Vogler 2003) and several *Rhinanthoideae* species (Kwak 1979). Regular biotic (cross) pollination is crucial for reproduction through seeds of many facultative selfing plant species, and autonomous selfing will only bridge temporal pollinator absence. Sometimes plant species that are believed to be autonomous selfers, e.g. *Arabidopsis thaliana*, or even apomictic plants, e.g. *Hieracium* species, benefit from facultative cross-pollination (Weeda *et al*. 1985; Hoffmann *et al*. 2003).

Degree of specialisation

The least specialised plant species were visited by many more insect species than the most specialised plant species: respectively 123 vs. 2 insect species in total, or 12.7 vs. 1.1 per site per day (chapter 2). The majority of the studied plant species can be regarded as generalist, and only a third is specialist. This is in concordance with literature stating that the majority of plant-pollinator interactions is of a generalist nature (Jordano 1987; Ellis & Ellis-Adam 1993; Waser *et al*. 1996; Memmott 1999). The asymmetric distribution of interactions between plants and flower visitors, i.e. specialist plant species are visited by generalist insects, and specialist insects visit generalist plants (Jordano 1987; Bronstein 1995; Waser *et al*. 1996; Memmott 1999; Olesen & Jordano 2002), was also found in this study (chapters 2 & 4). Generalist plants with mainly generalist visitors are in the majority, and of the specialist plants the majority are visited by generalist insects. Only two specialist plants are mainly visited by specialist visitors. One-to-one relationships between single plant and animal species are extremely rare, particularly in temperate climates (Kwak *et al*. 1998). The only case of such a tight bond in my study area is that between *Lysimachia vulgaris* and *Macropis europaea*. *Campanula rotundifolia* was mainly pollinated by the oligolectic bee *Melitta haemorrhoidalis*, but also by bumblebees. This plant species potentially has several pollinator species, mainly (oligolectic) bees (see chapter 6).

The vulnerability to pollinator loss is related to the degree of specialisation (chapter 6): pollinators of very common and extremely generalised plant species can be missed, as there will always be some visitors present acting as pollinators. This is for example the case for the Apiaceae species. However, plant species with few specialist pollinator species, *L. vulgaris* and *C. rotundifolia* in my study, hardly have any alternatives. For the remaining generalist and specialist, common and rare plant species with generalist pollinators it is currently difficult to predict the vulnerability to pollinator loss.

Plant population size

For generalist plant species, population characteristics like size and density are likely to be the most important "context" aspects determining the role of biodiversity for pollination. For *Succisa pratenis* (chapters 6 &7), population size rather than plant or insect diversity was the most important factor determining pollination quality (purity and amount of deposited pollen) and seed set. Small and diffuse plant populations can either not be found by insects, or insects ignore them as they do not offer enough resources (Kunin 1993; Ågren 1996; Kwak *et al*. 1998; Bosch & Waser 1999; Luijten *et al*. 2000; Mustajärvi *et al*. 2001). Biodiversity may become important in these cases. Plant diversity is either positive (facilitation) or negative (competition). The presence of other plant species can increase the visitation rate (Thomson 1978; Schemske 1981; Kwak 1988; Laverty 1991). This was observed for Asteraceae species (Thomson 1978). Small populations of sexually reproducing yellow Asteraceae, like *Leontodon autumnalis* or *Hypochaeris radicata* in this thesis, may profit from apomictic yellow Asteraceae. Abundant *Hieracium* species can provide a bulk food supply for the pollinators, locally oligolectic solitary bees. As they may make no difference between the plant species (see above), their inconstancy can be beneficial. Similarly, *Campanula rotundifolia* may benefit from *Jasione montana*, as I have occasionally observed that *Melitta haemorrhoidalis* visited *J. montana*.

A negative effect of diversity is a lower pollination quality, because of pollen loss and heterospecific pollen deposition (chapters 5 & 7). A large population enables insects to be flower-constant, increasing the rate of conspecific pollen deposition and decreasing pollen loss. Furthermore, small populations often suffer from inbreeding (van Treuren *et al*. 1993; Oostermeijer *et al*. 2000; Velterop 2000; Luijten 2001; Mustajärvi *et al*. 2001), due to among others increased geitonogamy (de Jong *et al*. 1993). For example, for *Scabiosa columbaria* a distance of 25 m between two patches reduces pollen flow by 75%, and 200 m distance by more than 99% (Velterop 2000). Similar results were obtained for *Succisa pratensis* (Kwak, unpub.).

Thus a small population size in combination with isolation affects pollination, and enhances negative genetic effects. However, a bad pollination may be better than no pollination at all. Sometimes small populations can be even advantageous: in small Salvia pratensis populations seed set is higher, because its pollinators (medium and long-tongued bumblebees) prefer low flower densities and smaller populations (Kwak *et al*. 1996).

Plant species level: summary

The importance of the diversity of flowering plants and flower-visiting insects for the reproduction and maintenance of a plant species depends on the context: the composition of the flower neighbourhood of the target species, the plant's pollination biology and life history (chapter 6), the degree of specialisation (chapters 4 & 6) and plant population characteristics, i.e. size, isolation and density (chapters 6 & 7). Other authors found little evidence that separate variables of pollination biology or plant breeding systems are related to the rarity of a plant species (Weller 1995) or susceptibility to habitat fragmentation (Aizen *et al*. 2002). Indeed, the rarity of a plant species in the Netherlands could only be predicted and explained with a combination of variables (Bekker & Kwak 2005; Kwak & Bekker 2005). In the latter studies, seed production, breeding system, clonality and soil seed bank longevity together significantly separated rare and common plant species. Rare and common plant species in the Netherlands did not differ in their breeding system (i.e., flower features, degree of selfing and specialisation of the pollinators). This means that the type of hypothesis about the role of biodiversity for pollination of individual plant species or populations must be of the context-dependence type. Predicting the vulnerability of single plant species and populations to loss of biodiversity, particularly pollinators, can thus only be done by combining species- and site-specific data.

POLLINATION AND BIODIVERSITY: THE PLANT COMMUNITY

Community aspects

At community level the point of interest is not the pollination of individual plant species, but the pollination of all species in the community. For this discussion about pollination at community level the aim of conservation matters, and I will assume that the aim is plant communities with a high diversity. Communities consist of common and rare species, dominant (core) and subdominant species, and the plant species are often arranged in patches. Here I interpret these aspects in terms of flowers rather than plant cover.

The communities at the sites in this thesis varied in plant diversity between 9 and 35 species, and flower-visiting insect diversity varied between 10 and 89 species (chapter 3). Core flower species at most sites belonged to Apiaceae (*Anthriscus sylvestris* and

Heracleum sphondylium), yellow Asteraceae (*Taraxacum officinale, Hieracium laevigatum, Hypochaeris radicata* and *Leontodon autumnalis*), Ranunculaceae (*Ranunculus repens* and *R. acris*), Lamiaceae (*Glechoma hederacea*) and Fabaceae (*Trifolium repens, T. pratense* and *Lotus corniculatus*). Other species could also be dominant locally. Endangered or Red-Listed species (sometimes locally abundant) were *Succisa pratensis* (Dipsacaceae), *Rhinanthus angustifolius* (Scrophulariaceae), and *Phyteuma spicatum* subsp. *nigrum* (Campanulaceae). The dominant (most abundant) flower visitors were discussed in chapter 8, and belonged to flies (Diptera: Syrphidae, Calyptrata and Bibionidae) and bumblebees. Less abundant flower visitors were beetles, butterflies, solitary bees and wasps.

A plant community as a whole can increase the number of flower-visiting insect species, and thereby the chance for appropriate pollinators (Corbet 1997). The chance that plant species are visited by effective pollinators may increase as insect species richness is higher (Corbet 1997). Species richness and abundance of plants and some insect groups (particularly bees) were positively affected by the diversity at landscape scale (i.e. type and intensity of land use and the related floral and structural diversity), and insect diversity was positively related to plant diversity within sites (chapter 3). Thus a positive effect of a higher plant diversity is that it increases total insect diversity and abundance (Bäckman & Tiainen 2002; Collinge *et al*. 2003; Potts *et al*. 2003; Armbrecht *et al*. 2004). However, the number of visitor species per plant species (plant linkage level) gives a more complicated picture. The direct effect of plant species richness on linkage level is negative, probably because insects are distributed more over the plant species (chapter 4). But the indirect effect through insect species richness is positive, reducing the negative effect considerably, and also a higher flower abundance strongly increases plant linkage levels. This may explain why in chapter 5, where linkage levels were analysed for only six plant species, plant diversity affected linkage level either positive or not. Also the high generalisation level of those six plants may be a reason for that. As discussed before, the data are based on frequencies, whereas the number of visits and the pollen loads may provide better insight in what happens to the pollination of the community. Analysing this for all species at so many sites will a very labour-intensive task (Memmott 1999; Forup & Memmott 2005).

The role of insect diversity for pollination at community level was extensively discussed in chapter 8. Even though only ten abundant insect species (2.5% of the species observed) constituted more than 50% of the visitors of two thirds of the plants, this is not enough for a guaranteed pollination of the *entire* plant community. Firstly, some of these visitors are not or only low-quality pollinators. Secondly, many more pollinator species are needed for the pollination of the plant community for reasons involving degree of specialisation, spatio-temporal variation, plant population structure, and interactions between plants (chapter 8, and see also above).

Relation between plant and pollinator declines

One of the remaining questions in this thesis is about the effect of the declining insect species groups mentioned in chapter 1 (butterflies and bees) on the pollination of the plants in the research area. The results presented in chapter 3, i.e. only common butterfly species, a strong effect of landscape and plant-species richness on solitary bees, and a lower abundance of long-tongued bumblebees compared to short-tongued species, are well in line with the Red-Listed status and general tendencies of these taxa (Kwak 1994b; Peeters & Reemer 2003; CBS *et al*. 2004; Goulson *et al*. 2005). Butterflies are generally relatively poor-quality pollinators in northern Europe (Jennersten 1984; Velterop 2000), thus their role in general patterns of plant species decline will be negligible. It is more likely that it is the other way around, as many butterflies are food specialists in the larval stage (van Swaay & Warren 2001; Thomas *et al*. 2004).

Solitary bees can be effective pollinators (Strickler 1979; Cane & Payne 1988; Velterop 2000; and see for example table 9.2 concerning foraging speed). The decline of solitary bees may therefore be detrimental for plant communities (Williams 1995; Buchmann & Nabhan 1996; Cane 2001). But vulnerable plant species depending on (oligolectic) bees are a small minority (chapters 6 & 8), and the asymmetric distribution of interactions makes that most of the plant species that are visited by solitary bees also have other visitors or pollinators (see above). Even more, the one plant species most dependent on a solitary bee, *Lysimachia vulgaris*, has increased steeply in the past decades (Stichting Werkgroep Florakartering Drenthe 1999; Tamis *et al*. 2004) and its bee is common in the Netherlands (Peeters *et al*. 1999). *Campanula rotundifolia* is declining slightly, probably due to habitat loss and nitrogen deposition (Plate *et al*. 1992; Stichting Werkgroep Florakartering Drenthe 1999; Tamis *et al*. 2004), rather than due to changes in the bee fauna: *Melitta haemorrhoidalis* is common (Peeters *et al*. 1999) and it was observed in most C. rotundifolia populations in the area. Other *Campanula* species have declined in the Netherlands (Plate *et al*. 1992; Tamis *et al*. 2004) and also some oligolectic bee species specialised on *Campanulaceae* (Peeters & Reemer 2003). The host plants of several other declining or extinct oligolectic bees have also declined (Plate *et al*. 1992; Peeters *et al*. 1999; Peeters & Reemer 2003; Tamis *et al*. 2004), but how these two are related is unknown and currently a complicated chicken-and-egg question. Firstly, most of the plant species are generalists, like the Dipsacaceae that have several alternative pollinators (chapter 7; Velterop 2000). The decline of many plant species is mainly subscribed to habitat deterioration, nitrogen deposition and inbreeding, for example for *S. pratensis* (Hooftman *et al*. 2003, 2004; Vergeer *et al*. 2003a & b). The decline of the bees can also be subscribed to habitat deterioration and a decline in habitat heterogeneity (chapter 8; Westrich 1996; Calabuig 2000; Cane 2001). Even though the decline of an oligolectic bee is not the immediate cause of the decline of the host plant, it can contribute to an acceleration of the plant's decline (chapter 7). Also, the decline of the pollinator can be a bioindicator of the state of a habitat (Kevan 1999) and be a bad omen of what is going to happen to the plant (chapter 7).

Dominant flower visitors in the study area of this thesis are generalist large syrphid flies characteristic of nutrient-rich habitats and bumblebees (chapter 8). Memmott *et al*. (2004) have analysed two data sets from the 1920s in the United States, where solitary bees had a very important role in the system. It would be interesting to investigate the same area as the 1920s again to see whether a shift to more generalist pollinators has happened there, and whether this is comparable to the current situation in western Europe.

There are many factors that primarily drive the decline of plant species, such as eutrophication and pollution (Andreasen *et al*. 1996; CBS *et al*. 2004), changes in hydrology (Bakker & Olff 1992; Grootjans *et al*. 2002), fragmentation and isolation of populations (Kwak *et al*. 1998; Velterop 2000; Hooftman & Diemer 2002; Hooftman *et al*. 2003; Vergeer *et al*. 2003b), or disruption of seed dispersal and seed bank processes (Bakker *et al*. 1996; Nathan & Muller-Landau 2000; Geertsema 2002; Blomqvist *et al*. 2003a; Soons 2003). Changes in pollination will come on top of this, thereby enhancing plant species decline. In agricultural landscapes, plant populations and communities are rather small and restricted to small, mostly linear habitats (Schaffers 2000; Geertsema 2002; Blomqvist *et al*. 2003b). Population and patch size are one of the key factors affecting pollination processes, like for *Succisa pratensis* (chapters 6 & 7) and *Phyteuma spicatum* (Kwak 1994b; Kwak & Vervoort 2000) in the research area. A high enough insect diversity may help plant species to reproduce in these small habitats.

Plant community: hypothesis and conclusion

Now I will turn to what type of hypothesis may be applicable to the role of biodiversity for pollination at community level. The applicability of a redundancy-type hypothesis (species are equal) is unlikely: many flower visiting insects occur in too low numbers to be important pollinators. Furthermore, it was shown that most plants have several alternative pollinators, effects of pollinator loss depend on which insect species disappear, and the pollination web has a stabilising effect. A keystone-species hypothesis may be more likely, but then a community will need many keystone species for the pollination of all plant species (chapter 8). For many ecosystem processes, including plant-pollinator interactions, it is more important that trophic interactions such as pollination take place, rather than which exact species is eating or pollinating which other species (Forup & Memmott 2005). An example is the relation between ants and trees: not some specific tree species, but the diversity of the trees as such explained ant diversity (Armbrecht *et al*. 2004). Similarly for keystone species, it is not so much the species per se, but the keystone role it plays in an ecosystem that matters (Mills *et al*. 1993). This means that the functional diversity is more important than species diversity for species interactions and diversity relations (cf. de Ruiter *et al*. 1995; Petchey 2004).

At the level of functional types, a keystone-species hypothesis may be useful: a minimal level of functional types of pollinators is required. However, spatio-temporal variation and seasonal effects require several pollinator species of a functional type: there can be a critical level of pollinator diversity (Neff & Simpson 1993). The continuous availability of a variety flower types is needed for maintaining a diverse pollinator assemblage, for example late-flowering species also depend on early species (see above). Also here a minimal level of functional (flower) types is required. Exceptional are strict specialists that will need their specific host or partner (e.g. monolectic bees, butterfly larvae and specialist plants). Some context dependence is also possible, for example habitat size or the type of ecosystem. It seems likely that the role of biodiversity lies somewhere between all species and some keystone-species. Hence, I propose that a good name for such a hypothesis will be "critical diversity-level hypothesis".

The data from this thesis showed how the diversity of plants and insects are related to the landscape and each other, and provide information about interactions between plant species and an estimate of the vulnerability of plant species to pollinator loss. To show a relation between the decline of bees and a decline of plants, other data are needed: for example, species richness and abundance of bees and bee-pollinated plants from various locations in combination with measurements of seed set and possible pollination deficiency. Sites with high and low bee diversity should be compared. To my knowledge such an extensive study has not yet been performed.

LANDSCAPE DIVERSITY, HABITAT FRAGMENTATION AND LONG-TERM EFFECTS

Habitat fragmentation and landscape heterogeneity

Habitat fragmentation is one of the major causes for species decline (Fahrig 2003) that also affects pollination by reducing pollen and gene flow, and reproductive output (Sih & Baltus 1987; Jennersten 1988; Rathcke & Jules 1993; Kwak *et al*. 1998; Steffan-Dewenter & Tscharntke 1999; Velterop 2000; Luijten 2001; Donaldson *et al*. 2002; Tomimatsu & Ohara 2002). The different types of land use in the area (chapters 1 & 3), intensive agriculture, grasslands and mixture of land-use types, and nature reserves and other natural areas, can also be regarded in terms of habitat fragmentation. The reserve areas are the most natural and heterogeneous, and the areas with intensive agriculture the most fragmented. In some parts of the area, particularly in the west, where agriculture is extremely intensive (Geertsema 2002), the structural diversity was almost zero: there are very few trees and hardly any bushes or hedges, while in the other landscape types there are (much) higher proportions of structural elements.

Insects responded at different spatial scales than plants did: landscape diversity had stronger effects on plants than on insects, and within insects it was strongest on insects with the smallest home range, i.e. solitary bees (chapter 3). Insects have different spatial (larger) and temporal (shorter) scales than plants (Sowig 1989; Sutherland *et al*. 1999), and also between insect groups and species there are considerable differences (Chust *et al*. 2003, 2004; Samways 2005). Therefore effects of landscape and habitat fragmentation differ between plants and insects (Vessby *et al*. 2002; Dauber *et al*. 2003; Chust *et al*. 2004). An organism's perception of whether something is a boundary or not, or whether it is sharp or diffuse, is an essential parameter in understanding the effects of habitat fragmentation (Chust *et al*. 2004). Some small insects can disperse surprisingly well in a fragmented landscape, e.g. common parasitoid wasp species (Elzinga 2005). But many rare or endangered insect species are resident, for which strongly modified habitats are barriers (Samways 2005); this is most well known for butterflies (Hill & Fox 2003; Stefanescu *et al*. 2004). The dispersal of rare species from reserve areas to neighbouring intensified agricultural areas was very low in our study. The only example is the relatively rare syrphid fly *Eristalis anthophorina*, a species typical for acidic fens and other wetland areas (Achterkamp *et al*. 1998; Speight *et al*. 2001). It was observed at sites within the most intensively used agricultural areas, that lie within 1 km from the

Netherlands' largest bog reserve, the Fochteloërveen (van der Heiden *et al*. 2005). The diversity of bees is low in agrarian landscapes where all semi-natural habitats have been removed, and where no core habitats such as pastures or forests exist in the vicinity (Calabuig 2000; Kleijn *et al*. 2001). Habitat heterogeneity is an important aspect for insects in fulfilling their life cycle (Bronstein 1995; Verberk *et al*. 2002; Chust *et al*. 2003; Samways 2005). Several "partial habitats" are needed within one ecosystem: sites for offspring or nesting, mating and foraging can be different (Westrich 1996).

A high plant diversity in linear habitats may function as corridors between plant populations and patches, as insects will move more willingly along flower-diverse corridors (Saville *et al*. 1997; Kwak *et al*. 1998; Kwak & Vervoort 2000; Velterop 2000; Osborne & Williams 2001). In this way the effects of fragmentation for both plants and insects may be reduced. But also simple and unexpected structures in a landscape can guide insects through an open landscape: bumblebees were observed to make use of ditches and even construction tape and barbed wire (L. Cranmer, pers. comm.). However, what is a corridor for one pollinator species is not one for another (Samways 2005). This depends on e.g. plant species composition and structural aspects like vegetation height and corridor width.

Long-term effects and landscape history

The long-term effect of biodiversity and heterogeneity at landscape scale on pollination is difficult to discover. One reason is species specificity. Another reason is that the effects are often delayed: pollinators mostly disappear before the plant, and effects of shifts of pollinator assemblages on extinction may be delayed by clonality and long life spans, or blurred by annual variation (Corbet 1997; Johnson & Steiner 2000; Spira 2001; van Rossum *et al*. 2002). Furthermore, simplified systems that have lost some of their pollinator species still have pollinators, but may be very vulnerable to even further change (Waser *et al*. 1996).

Other long-term effects go into a different direction, i.e. back into the past. Site-specific historical effects can go back as far as several millennia (Lindborg & Erikson 2004). In one area in Sweden, plant species distribution was not related to current spatial structures, but to historical landscape connectivity of 50 to 100 years ago (Lindborg & Erikson 2004). The landscape in the Dutch province of Drenthe, in which the research area is situated, was inhabited since the end of the last glacial period, and since the New Stone Age, agriculture and large-scale deforestation were increasing (Spek 2004). The image of the region used to be very romantic, viewed as a stable area where "time has stood still". Recently, this image proved to be false: since the Iron Age land use practices have changed repeatedly (Spek 2004). A reconstructed paleogeographic map from ca. 1000 AD shows that most of the research area used to be shallow brook valleys and boulder-clay areas with partially forests and meadows. Another part used to be raised-bog and bog-edge vegetation. During the Middle Ages the land use changed more than before, when meadows were turned into pastures, deforestation maximised and bogs were declining (Spek 2004). Still, many areas were species-rich with a high landscape heterogeneity. In the twentieth century the landscape was severely affected by agricultural intensification and abandonment of traditional agricultural practices that had led to species-rich vegetations. Only since the 1970s restoration and conservation of remnants of the brook valley meadows and raised bogs took place (Bakker & Olff 1992; Grootjans *et al*. 2002; van der Heiden *et al*. 2005). Most of the species-rich sites are in the brook valley area, whereas many species-poor sites are in the intensive agriculture area in the west that used to be a bog-edge and raised bog. Whether this will have affected the results in this thesis is unknown. Nevertheless, there is considerable overlap of core (plant) species at most sites. In this thesis, short-term mechanisms of biodiversity effects on pollination have been shown. These processes can have long-term consequences, while the mechanisms behind them are (at least partially) short-term.

MANAGEMENT AND CONSERVATION

Mowing, flowering and visitation

When a vegetation is mown, the standing crop is removed, including buds, flowers or unripe fruits. Reproduction is thus inhibited. Many plant species will produce new shoots after mowing, and may also produce flowers for a new chance of reproduction. Not all plant species can do this equally well, and the amount of regrowth and reflowering also depends on the timing, frequency (how often) and intensity (how much is removed) of mowing (Londo 1974; Bakker *et al*. 1980; Bakker 1989; Broyer & LauransonBroyer 1996; Grootjans *et al*. 2002). The flower-visiting assemblage and the amount of visitation of reflowering plants may differ before and after mowing, caused by the phenology of insects. This may also affect the pollination of the plants.

Mowing of road verges and ditch banks is a normal management measure. This has also been applied in the sites of this thesis. In 2000 and 2001 the management and mowing regimes were extremely variable between sites (chapters 2,3 and 4). It was not the goal of this thesis to analyse the effect of mowing on pollination, but due to its frequency and variability it cannot be ignored. No effects of mowing regime on plant species richness, flower abundance and total insect species richness and abundance were found, only bees were affected (chapter 3). This is mainly due to the fact that in cases when there were no flowers after mowing, insects have not been monitored and were not included in the analyses.

An overview of the management of the sites in the area, and available data to illustrate the amount of reflowering after mowing and effects of second or delayed flowering on flower visitation are presented in box 9.1. The majority of the plant species analysed (71%) was able to produce new flowers, but (much) fewer than before. The flower visiting assemblage and the amount of visitation of reflowering plants before and after mowing differed only for generalist plant species.The visitor assemblages of the specialist plants hardly differed. For all species the number of visitors was mostly lower later in the season, which may only partly be due to a lower number of flowers. In what way this may affect pollination and therefore the reproductive output of a delayed or second flowering cannot be said from the data in this thesis, but I will point out a few problems.

When a plant flowers later than the flower visitor flies, they may both have a problem. A "phenological mismatch" of plants and their pollinators due to mowing is most likely for plants depending on few pollinators with a limited phenological time span (Bronstein

1995). *Campanula rotundifolia* is such a plant species; in the research area it depends on mainly oligolectic solitary bees (see above). In the Netherlands, the flowering phenology of the plant can be very long, between June and autumn (van der Meijden *et al*. 1996), but its main pollinators, females of the oligolectic bee *Melitta haemorrhoidalis* (chapter 7), have their peak in the second half of July and first half of August (Peeters *et al*. 1999). The phenology of both plant and bee in the research area (between 2000 and 2003) are well in line with literature: *C. rotundifolia* was flowering from the beginning of June until the beginning of October (peak in August), and *M. haemorrhoidalis* was observed from the second half of July until the end of August (peak in last week of July and first week of August). The plant can produce new flowers after mowing (box 9.1), and the observed second flowering was still within the flight period of the bee. Thus the mowing at this particular site will not have had negative effects on pollination. However, later mowing and reflowering when the bee is absent will reduce the reproductive output severely. Without the bee seed set is very low (chapter 6).

Mowing itself can also have short-term evolutionary consequences if the same regime is used for several years or decades. The difference in flowering phenology between plant populations can depend on the timing of mowing, e.g. in *Rhinanthus angustifolius* (ter Borg 1972), *Centaurea nigra* and *Succisa pratensis* (Lack in Proctor *et al*. 1996).

The effect of the removal of flowers on the visitors depends on how long no food is available, whether alternative sources are nearby, and whether insects are able to migrate or forage on larger distances. For species with a strong site constancy behaviour, e.g. bumblebees (Osborne & Williams 2001), the removal of flowers can have negative effects on colony growth (Thomson *et al*. 1997). When flowers are removed, insects can either wait, migrate or die, and when mowing is too frequent and the time before new flowers are produced is very long, some species may even go extinct in a certain area (Fussell & Corbet 1992; Osborne & Corbet 1994). Some parts of the research area where several verges were mown within the same day or week could be without any flowers for a week or more. In 2000, one site even had only flowers in the beginning of the season. At sites in continuous agricultural areas there were no alternative food sources in the time after mowing for the insects, as there were no flowers in the crop fields or intensively used grasslands. The effect of no alternative food sources was apparent for the short-distance central-place foragers (see above): the number of solitary bee species and individuals was significantly higher in unmown or mown sites with unmown patches or meadows in the vicinity, compared to mown sites without alternative foraging possibilities (chapter 3).

Management of road verges and ditch banks

In many road verges mowing was very frequent and intensive in the area, however, for the majority it was only once or twice (box 9.1). Nutrients (N and P) accumulate if the hay is not removed within two weeks after mowing, leading to species-poor plant communities with mainly fast-growing grasses and other eutrophilous plant species like *Anthriscus sylvestris* or *Urtica dioica* (Schaffers 2002). *Anthriscus sylvestris* was extremely dominant, and could set seed at many sites before the first mowing. The species showed a dramatic increase in the research area, also benefiting from increased nitrogen deposition caused by application of manure and artificial fertilisers in agriculture (Stichting Werkgroep Florakartering Drenthe 1999). It is considered as problematic for both agriculture and conservation (van Mierlo & van Groenendael 1991; Hansson & Persson 1994).

An example of a rare and declining species that is extremely sensitive to high nutrient levels is *Succisa pratensis* (Bühler & Schmid 2001; Soons 2003; Vergeer *et al*. 2003a). In 2000, *S. pratensis* occurred at five sites in the research area, in 2003 this was reduced to three. The sites were intensively searched for non-flowering rosettes, but none were found. The two disappeared populations were small, but only mown once late in the season. What exactly caused the disappearance cannot be said. At one of the three sites where *S. pratensis* remained, the topsoil was removed (box 9.1). After topsoil removal, only few plants remained at the ditch side of that verge, but were mown off before they could set seed (chapter 7). Not far away from that site (< 2 km) is a road verge with an ecological management regime (box 9.1), but without any rare species. It would be a good idea to include the site with the *S. pratensis* population in such a regime, or introduce the species with seeds from the declining population. In this way the number of *S. pratensis* populations can be increased using genetic material from within the same area.

So far it may appear that mowing is only negative, but of course the vegetation of the grassland-like road verge and ditch habitats needs to be mown for reasons of limiting vegetation succession. Mowing can reduce nutrient contents and change species interactions, and therefore is a normal type of management for restoring and conserving species-rich grassland habitats (Bakker *et al*. 1980; Bakker 1989; Bakker & Olff 1992; Wynhoff *et al*. 2001; Grootjans *et al*. 2002) and road verges (Schaffers 2002). It can even facilitate seed dispersal (Strykstra *et al*. 1997).

The type of management, as discussed above, is crucial. Which type of management is chosen depends on the conservation goals and other functions of a habitat. The vegetation near a road is kept short for traffic safety reasons, and in ditches for reasons of water management. In agricultural landscapes, farmers will not be too happy with potential pest species. Conflicts also arise within nature conservation: do we want to protect plants, bees, butterflies, grasshoppers or birds? These taxonomical groups and individual species can require contrasting management regimes (Verlaar 1990; Westrich 1996; Wynhoff *et al*. 2001; Griebeler & Seitz 2002; Samways 2005). The goals determine what criteria should be used. However, in parts of the research area any management goals apart from keeping the vegetation as short as possible seem to be lacking.

Criteria for conservation in agricultural landscapes: pollination

Food is the basis for human survival, and therefore agricultural landscapes will not disappear. Thus there will always be a need for finding ways in which agriculture and nature can be in harmony and may even be mutually beneficial. Furthermore, agricultural activities were the cause for many species-rich communities and ecosystems in Europe before intensification of agriculture (chapter 1). The importance of habitat remnants in agricultural landscapes for conservation is recognised by policy makers, such as the European Union and its member states (Kleijn *et al*. 1999; Manhoudt & de Snoo 2003; Kleijn & Sutherland 2004). This is also true for road verges and ditches (Schaffers 2000; Geertsema 2002;

Blomqvist 2005). What are criteria for management and mowing of these habitats? I will only focus on the point of view of pollination and plant-pollinator interactions.

The presence of pollinators is important for the plants' reproduction. Assuming that there is a critical diversity of pollinator species richness and plant species, a minimal plant diversity and flower availability are crucial for the conservation of flower-visiting insects (see above). For the conservation of plants, particularly in fragmented habitats, the habitat requirements of pollinators and the phenology of both flowering plants and their pollinators should be taken into consideration. Important pollinator groups are bumblebees, syrphids, flies and solitary bees. A recent questionnaire about conservation and insects among managers of Dutch nature reserves revealed that only in a minority of cases the major pollinators, bees and hoverflies, are included as target groups for management practices (Bulten & Kwak 2002). This indicates that more attention is needed for these groups, particularly when target species for conservation are insect-pollinated plants.

Rare plant species may indirectly depend on common species (Bronstein 1995), therefore also core and common plant species should be integrated in conservation (Dupont *et al*. 2003). For diverse plant communities it is crucial that litter is removed within one to two weeks after mowing (Schaffers 2002), and the machinery used should only mow the vegetation, and not destruct growing meristems, the root zone or destroy nesting sites of pollinators.

Furthermore, plants must have a chance to flower and set seed. This will determine the timing of mowing as it is species dependent. A solution for the problem of different phenologies of plant species can be to mow only parts of a road verge or ditch bank, or have a different timing for either side of a road or ditch. Mowing can be directed at certain target species. For example, in the nature reserve Elperstroom- De Reitma (chapters 6 & 7), large patches of *Succisa pratensis* (a target species for conservation in that area) were not mown by Dutch State Forestry until the plants had had a chance to set seed.

The habitat requirements of the insects have been discussed in chapter 8 and above. In summary, important are habitat heterogeneity, availability of nesting sites and food plants throughout the season, including late and early plants. These aspects will also determine the timing of mowing, together with the survival of some insect larvae.

Increasing the amount of habitat and its heterogeneity in agricultural landscapes is frequently stimulated by sown herb strips along fields or agri-environmental schemes. Sown herb strips have shown to increase insect diversity, including natural enemies of crop pests (Salveter 1998b). Agri-environmental schemes have shown to be only partially or not effective for increasing biodiversity, probably due to their short-term and scattered nature (Kleijn *et al*. 2001; Kleijn & Sutherland 2004; Blomqvist 2005). A better and more continuous management (mowing, coppicing, etc.) of small elements that are already present in agricultural landscapes, like the sites discussed in this thesis, may sometimes be cheaper than creating new, artificial and annual habitats.

In the end, the diversity of both plants and insects will be important for long-term insurance of plant species richness. Or as Corbet (1997) phrased it: "A diverse pollinator assemblage requires a diverse vegetation, and a diverse vegetation requires a diverse pollinator assemblage".

Box 9.1:

Effects of mowing on flowering and flower visitation

INTRODUCTION

Mowing of road verges and ditch banks is a regularly applied management measure, and has also been applied in the sites of this study. The consequences of mowing for the flowering of plants, insect visitation and pollination are largely unknown (see chapter 9 for discussion about management in general). In the previous chapters of this thesis mowing was regarded as a side effect. In this box I will do a more systematic analysis of the effect of mowing with the data that are available. The following questions are asked: (1) What are the mowing regimes in the research area? (2) How many and which plant species produce new flowers after mowing? (3) Do visitor compositions differ between first and second flowering?

MATERIAL AND METHODS

Data about management were gathered for the sites (road verges and ditch banks) in the research area in 2000 (42 sites) and 2003 (49 sites). From May to October the sites were inspected approximately every two weeks and the mowing regime was scored.

For the question of reflowering and the amount of reflowering the transect observations from 2000 were used (see chapter 3 for methods). From this data set nine sites were selected that were mown in either the end of May, in June or the beginning of July in 2000. Per plant species that was flowering at the time of mowing, the amount of reflowering was calculated as the percentage of the maximum number of flowers produced after mowing relative to the maximum number of flowers before mowing. Only plant species with a minimum of 25 flower units just before mowing were used to prevent outliers.

In addition, in 2003, the number of flower units (flowers, umbels, heads, stems) was scored per plant species approximately every ten days in seven permanent quadrates of 4 m^2 at five sites with an expected difference of mowing regime. From the data in the permanent quadrates, flowering phenology curves are produced. Flowering is expressed as percentage of flowers on the day with the maximum number of flowers (100%).

For analysing possible differences of visitor compositions before and after mowing, two generalist plant species, i.e. *Hypochaeris radicata* (two sites) and *Jasione montana* (one site), and two specialist species, i.e. *Symphytum officinale* and *Trifolium pratense* (both at two sites) were selected from the transect data of 2000. All selected sites were mown once in June. The flower abundance, the number of visitors, the number of visitor species and the composition of visitors from two censuses per site before mowing were compared with two censuses after mowing. The *Jaccard* similarity-index, the fraction of visitor species observed before and after mowing relative to all species observed, was used to estimate differences in visitor composition before and after mowing.

RESULTS AND DISCUSSION

Mowing regime

The mowing frequencies varied between 0 - 6 times per site in 2000, and between 0 - 3 times in 2003 (fig. 9.2). In both years the majority of sites was mown once or twice. Eventually also the remaining sites (mostly along arable fields) were mown after the flowering season in November. The earliest mowing was in May in both years. In 2000, most mowing events were in July and September. In 2003, most sites were mown in June, July, September and October, but in August no mowing was done. August 2003 was extremely dry and hot, therefore mowing was probably postponed until September. This may also have been a reason for why the mowing frequency was lower than in 2000. Apart from mowing, also other disturbing or management activities took place: at some places in both years (parts) of sites were destroyed by agricultural machines, and often the dead matter from cleaning ditches was cast on the ditch banks or adjacent verges. Locally, individual plants like *Anthriscus sylvestris* were chopped off or sprayed. In 2003, two sites were dug up for constructing new sewage systems and cables, and at two other sites the entire topsoil was removed.

At several sites, a narrow strip of vegetation at the remotest part from a road close to a ditch remained after mowing. Plants were still flowering in these remains. This may explain why no relation was found between mowing regime and flower abundance or species richness in chapter 3. Furthermore, sometimes only several hundreds of meters of a road verge or only one side of a road were mown. Litter removal was scored in 2003. At 5 out of the 41 mown sites litter was removed and at 36 sites it was not. Not removing litter can limit the regrowth of plants and may also be a cause of reduced reflowering. Furthermore, it leads to accumulation of nutrients, thereby affecting the vegetation composition on a longer term. The height of a vegetation was no criterion for mowing, as at some sites where vegetation height was already extremely low (e.g. 5 cm), mowing would still progress.

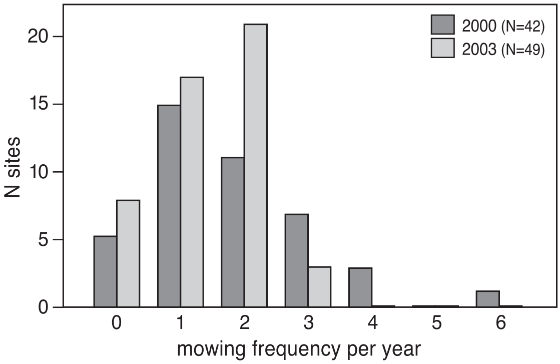


Fig. 9.2. Mowing frequencies of road verges and ditch banks between the beginning of April and the end of October in 2000 and 2003.

The type of mowing machinery used by the district authorities often reduced the vegetation, and sometimes even the soil, to dust. Rotary mowers were less often used. Many different parties were involved with the management, that were not all cooperating: districts, the province, water managing authorities, local land owners and farmers, and state forestry. It happened to occur that a site was repeatedly mown by different parties. At one site, there was a so-called "ecological road side management", i.e. the site was only mown once a year at the end of the season. This type of management surprisingly included stripping off the vegetation and parts of the top soil (in July 2003), but less severely than the other two sites where the entire topsoil was removed.

Reflowering

At the selected sites of the transect observations 28 plant species (3 annual or monocarp and 25 perennial) were flowering at the time of mowing (table 9.3). The majority (20) produced new flowers after mowing, but most of them (16) less or much less than before mowing. Reflowering does not seem to be related to plant family or flower type, but is species-specific. Many (13) plant species occurred at only 1 site at the time of mowing, making it difficult to generalise.

The flowering curves from the permanent quadrates (2003) differ between the sites (fig. 9.3), which is only partially due to different times of mowing. Local phenological variation and species composition are other causes, as shown by the quadrates 19G, 19K, 20A and 35A, that were all mown on July 16th. Site 11 is one of the road verges in which the entire topsoil was removed in July, therefore hardly any reflowering could take place. In quadrate 35A flowers were not reduced to zero. The plants still flowering were *Potentilla reptans* and *Trifolium repens*, that were not cut by the mowing machinery due to their low height. All sites were mown late in the season in September or October. Contrary to early mowing, after this hardly any reflowering took place, apart from some flower heads of *Leontodon autumnalis*.

The effect of mowing is very nicely illustrated at site 22: quadrate 22A was mown in July and September, whereas 22B only in September. Because of the similarity of the vegetation in those quadrates, the flowering curves of individual species flowering at the time of mowing are shown in figure 9.4. In 22A there is a gap in flowering, while in 22B it is continuous. In 22A, five perennial plant species produced new flowers after mowing. The only annual, *Rhinanthus angustifolius*, did not reflower, strengthening the expectation that annuals may less often reproduce new flowers than perennials. *Achillea millefolia* and *R. angustifolius* were both at the peak of flowering in 22B when mowing occurred in 22A. *Achillea millefolia* reproduced new flowers, but much less than before (20%). Also *Hypochaeris radicata* produced less flowers, but still its curve in 22A is similar to 22B. It had already passed its peak at the time of mowing, and maybe this species naturally produces extra flowers later in the season. Nevertheless, the reproductive output from the first peak in 22A was zero. *Hieracium laevigatum, H. umbellatum* and *Campanula rotundifolia* all started to flower at the time of mowing, resulting in delayed flowering curves. As a result, the length of the flowering period of *H. umbellatum* and *C. rotundifolia* is much shorter in 22A than in 22B.

Table. 9.3. Plant species affected by mowing at nine sites (road verges and ditch banks) in 2000. Sites were mown in either the end of May, June or the beginning of July. Per plant species the reflowering was calculated as the percentage of the maximum number of flowers produced after mowing relative to the maximum number of flowers before mowing. These percentages are averaged per species: Values of 0% mean that no new flowers were produced after mowing, below 100% that less flowers are produced than before mowing, and above 100% more flowers than before mowing.

Plant species	family	flower type	life history	% reflowering ±SE	N sites
Aegopodium podagraria	Apiaceae	umbel	perennial	0.0	1
Anthriscus sylvestris	Apiaceae	umbel	perennial/monocarp	0.0	1
Bellis perennis	Asteraceae	head	perennial	22.7 ±17.2	2
Crepis capillaris	Asteraceae	head	annual	82.5 ±51.4	2
Hieracium aurantiancum	Asteraceae	head	perennial	2.6	1
Hieracium laevigatum	Asteraceae	head	perennial	1300 ±770	5
Hypochaeris radicata	Asteraceae	head	perennial	264 ±127	8
Matricaria species	Asteraceae	head	annual	1.6 ±1.6	2
Taraxacum officinale	Asteraceae	head	perennial	1.1 ±1.1	2
Valeriana officinalis	Valerianaceae	brush	perennial	22.0 ±22.0	3
Cardamine pratensis	Brassicaceae	bowl	perennial	0.0	1
Rorippa amphibia	Brassicaceae	bowl	perennial	0.0	1
Lysimachia vulgaris	Primulaceae	bowl	perennial	0.0	2
Ranunculus acris	Ranunculaceae	bowl	perennial	15.4 ±7.0	2
Ranunculus repens	Ranunculaceae	bowl	perennial	3.8 ±3.5	4
Potentilla reptans	Rosaceae	bowl	perennial	0.0	1
Lychnis flos-cuculi	Caryophyllaceae	tube	perennial	8.1 ±7.6	3
Silene dioica	Caryophyllaceae	tube	perennial	5.0	1
Symphytum officinale	Boraginaceae	tube	perennial	7.6 ±7.6	2
Iris pseudacorus	Iridaceae	gullet	perennial	0.0	1
Glechoma hederacea	Lamiaceae	gullet	perennial	0.0	1
Lamium album	Lamiaceae	gullet	perennial	15.4	1
Linaria vulgaris	Scrophulariaceae	gullet	perennial	1125	1
Scrophularia nodosa	Scrophulariaceae	gullet	perennial	2.0	1
Lupinus polyphyllus	Fabaceae	flag	perennial	1.8	1
Trifolium pratense	Fabaceae	flag	perennial	54.2 ±22.9	5
Trifolium repens	Fabaceae	flag	perennial	325 ±127	6
Vicia sepium	Fabaceae	flag	perennial	4.7 ±4.7	3

Visitation

The amount of reflowering of the four plant species differed between sites: it could be more, less or in the same order of magnitude compared to before mowing (table 9.4). The number of visitors (per flower unit and absolute) after mowing was always less than before for both generalist species (*Hypochaeris radicata* and *Jasione montana*). For *Symphytum officinale* and *Trifolium pratense* there were either more or less visitors after mowing. The difference in visitor species and abundance can be due to phenology and differences in flower abundance (chapter 3).

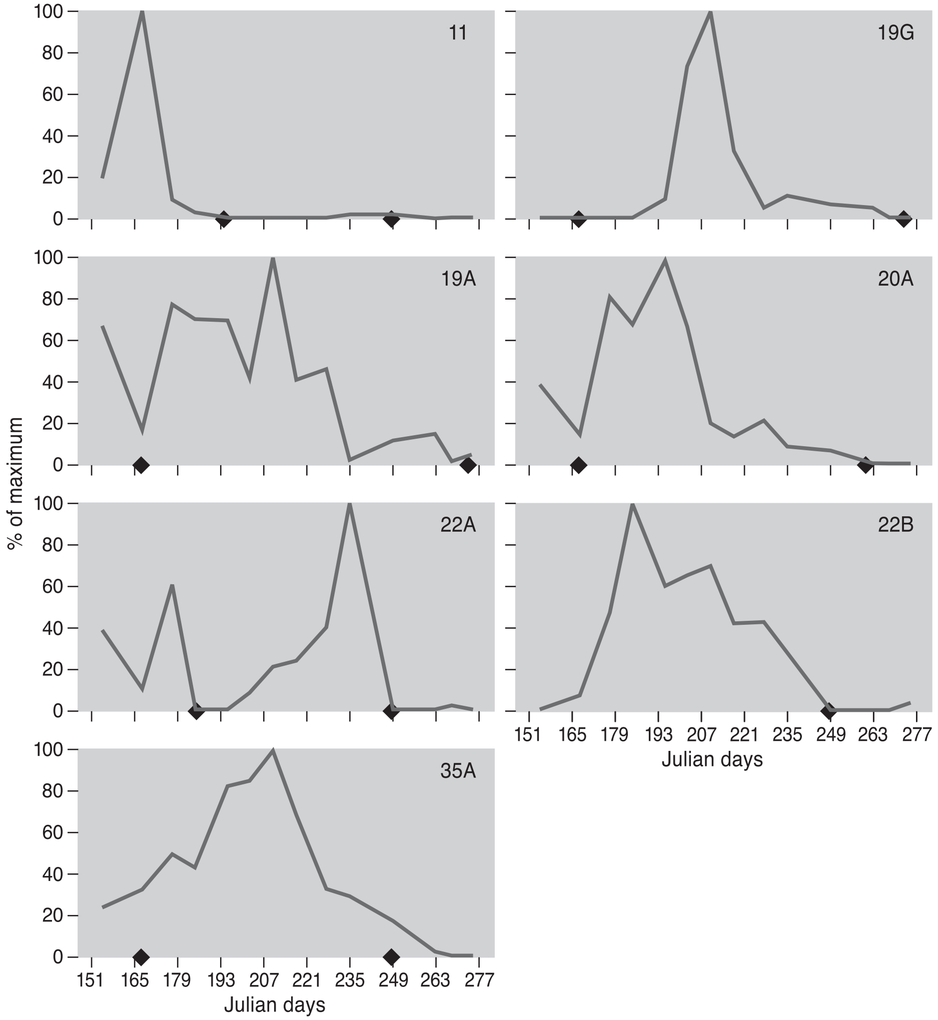


Fig. 9.3. Flowering phenology in road verges in relation to mowing in course of 2003 (in seven permanent quadrates of 4 m^2 at five sites). The flower abundance is shown as percentage of the maximum number of flowers of all plant species in a permanent quadrate (100%). Dates are expressed as Julian (continuous) days; day 151 is the June 1st, 2003. Mowing is indicated with diamonds on the x-axis. At two sites there were two permanent quadrates (19G and 19K, and 22A and 22B). The first mowing event at site 11 is actually the removing of the entire topsoil (ca. 5 cm) and not mowing.

Hypochaeris radicata and Jasione montana had different visitor compositions before and after mowing: the Jaccard-indices varied between 0.13 and 0.27. Also the main visitor species were different before and after for both plants (table 9.4). For the two specialist plant species there hardly was any difference before and after mowing. The Jaccard-indices were between 0.43 and 0.67, and the main visitors were always bumblebees (*Bombus* species), but sometimes in different proportions between the species.

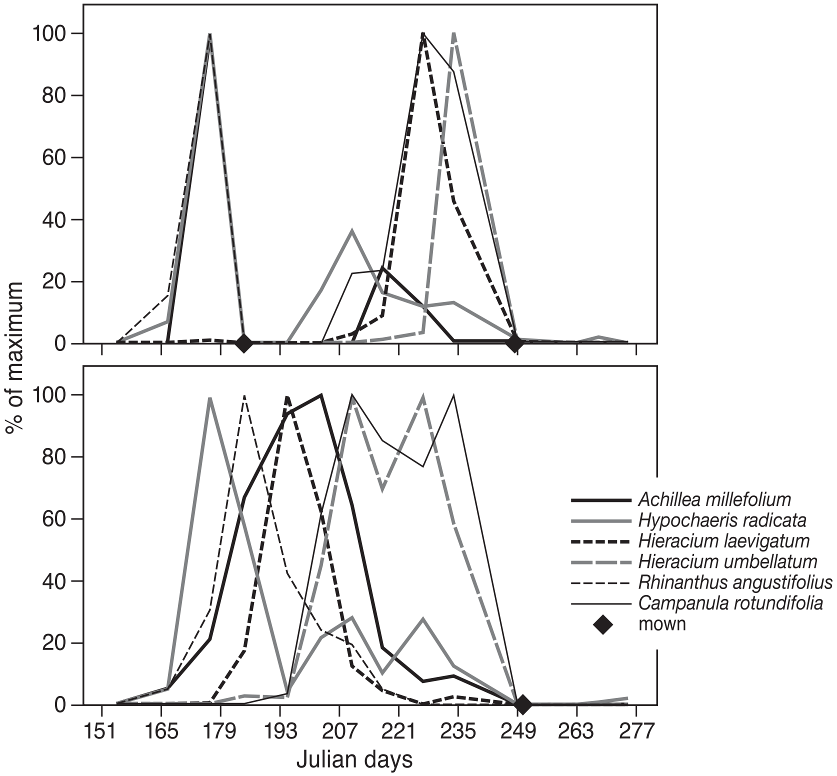


Fig. 9.4. Flowering phenology of six plant species in two permanent quadrates of 2x2 m in 2003. The permanent quadrates are in road verges at either side of a small road. The quadrate 22A (upper graph) was mown twice, 22B (lower graph) only once. The flower abundance is expressed as percentage of the maximum number of flowers of all plant species in a permanent quadrate (100%). Dates are expressed as Julian (continuous) days; day 151 is the June 1st, 2003. Mowing is indicated with diamonds on the x-axis.

Table. 9.4. Summary of the effects of mowing on the four plant species from table 9.2 and expectations for pollination and seed set. Signs indicate situation after mowing compared to before mowing: less (-), equal (=) or more (+).

	species	flowering	visitation	visitor species	effective visitors	expected pollination	expected seed set
generalist	*H. radicata*	-	-	-	-/=	-/=	-
	J. montana	+	-	-	-	-	-
specialist	*S. officinale*	+/-	+/-	=	=	=	=/-
	T. pratense	+/-	+/-	=	=	=	=/-

Conclusions

The mowing regime was highly variable in the area and in most road verges and ditch banks the litter was not removed. Many persons and authorities are managing the area, sometimes simultaneously. The majority of plant species analysed (71%) produced flowers after mowing in spring or summer, but mostly less than before mowing. The flower-less gap between mowing and reflowering at a site differed between some days and two weeks. After late mowing did hardly any plant species produce new flowers. For all four plant species there were differences in the number of visitor individuals before and after mowing. Visitor composition differed before and after mowing for the generalist plant species, and also when the number of flowers was higher after mowing, the number of visitors and visitor species was lower. For the specialist plant species the number of visitors was lower or higher after mowing, but the number of visitor species and the composition hardly differed. Whether the differences in visitor compositions between the plant species is also reflected in differences in pollination can only be guessed. Had the vegetation not been mown, it is likely that the generalist species would have had a higher pollination and seed set compared to a mown situation (table 9.3). For the specialist plants it would have been equal or higher. Reduced seed set after mowing caused by reduced flowering and pollination is better than no seed set at all, as the latter would mean no reproduction.

Table. 9.5. Visitation of four plant species early in the season before mowing ("pre") and later in the season after mowing when plants had produced new flowers ("post"). The sites were mown in June. Given are the flower abundance, the number of visitors per flower unit (the total number of observed individuals in brackets), the number of visitor species, the *Jaccard* similarity-index and the main visitor species (in order of frequency; expected **effective pollinators** in bold). The *Jaccard* similarity-index is the fraction of visitor species observed before and after mowing relative to all species observed.

Plant species		Flower abundance		N visitor individuals per flower unit		N visitor species		Jaccard	Main visitors	
		pre	post	pre	post	pre	post		pre	post
Generalists										
Hypochaeris radicata	site 1	1154	1013	0.038 (44)	0.033 (33)	19	14	0.27	***Lasioglossum leucozonium***, *Episyrphus balteatus*, ***Helophilus pendulus***	*Episyrphus balteatus*, ***Panurgus calcaratus***, *Syrphus ribesii*, ***Musca* spec**.
	site 2	5875	473	0.031 (180)	0.025 (12)	23	8	0.19	*Syrphus* spec., ***Eristalis intricarius***, ***Eristalis tenax***, *Episyrphus balteatus*	Small Tachinidae (cf. *Siphona* spec.)
Jasione montana	site 1	475	878	0.080 (38)	0.011 (10)	19	7	0.13	***Eristalis intricarius,*** ***Volucella bombylans,*** *Thymelicus lineola*	***Bombus terrestris,*** *Episyrphus balteatus*
Specialists										
Symphytum officinale	site 1	338	112	0.053 (18)	0.098 (11)	3	2	0.67	***Bombus pascuorum***	***Bombus pascuorum***
	site 4	760	1225	0.030 (23)	0.024 (30)	5	5	0.43	***Bombus pascuorum***, ***B. terrestris***	***Bombus terrestris***, ***B. pascuorum***
Trifolium pratense	site 1	1059	1290	0.008 (8)	0.004 (5)	3	3	0.50	***Bombus pascuorum*** ***B. hortorum***	***Bombus pascuorum***, ***B. terrestris***,*Thymelicus lineola*
	site 5	459	339	0.013 (6)	0.047 (16)	3	3	0.50	***Bombus pascuorum***, ***B. terrestris***	***Bombus pascuorum***

References

Achterkamp,B., van Grunsven,R., Lankreijer,L., van der Linden,J., Reemer,M., van Steenis,W., Termaat,T., Wakkie,B. & Zeegers,T. 1998. Voorlopige atlas van Nederlandse zweefvliegen (Syrphidae). Nederlandse Jeugdbond voor Natuurstudie (NJN); European Invertabrate Survey- Nederland, 's-Graveland/ Leiden, the Netherlands.

Adams,A.W. 1955. *Succisa pratensis* Moench. (*Scabiosa succisa* L.). Journal of Ecology 43: 709-718.

Ågren,J. 1996. Population size, pollinator limitation, and seed set in the self-incompatible herb *Lythrum salicaria*. Ecology 77: 1779-1790.

Aizen,M.A., Ashworth,L. & Galetto,L. 2002. Reproductive success in fragmented habitats: do compatibility systems and pollination specialisation matter? Journal of Vegetation Science 13: 885-892.

Andreasen,C., Stryhn,H. & Streibig,J.C. 1996. Decline of the Flora in Danish arable lands. Journal of Applied Ecology 33: 619-626.

Armbrecht,I., Perfecto,I. & Vandermeer,J. 2004. Enigmatic biodiversity correlations: ant diversity responds to diverse resources. Science 304: 284-286.

Armbuster,W.S. & McGuire,A.D. 1991. Experimental assessment of reproductive interactions between sympatric *Aster* and *Erigeron* (Asteraceae) in interior Alaska. American Journal of Botany 78: 1449-1457.

Armbuster,W.S., Fenster,C.B. & Dudash,M. 2000. Pollination "principles" revisited: specialization, pollination syndromes, and the evolution of flowers. Det Norske Videnskaps Akademi. I. Matematisk-Naturvidenskapelige Klasse, Skrifter, Ny Serie 39: 179-200.

Ashman,T.-L. & Stanton,M. 1991. Seasonal variation in pollination dynamics of the sexually dimorphic *Sidalcea oregana* ssp. *spicata* (Malvaceae). Ecology 72: 993-1003.

Ashworth,L., Aguilar,R., Galetto,L. & Aizen,M.A. 2004. Why do pollination generalist and specialist plant species show similar reproductive susceptability to habitat fragmentation? Journal of Ecology 92: 717-719.

Bäckman,J.-P.C. & Tiainen,J. 2002. Habitat quality of field margins in a Finnish farmland area for bumblebees (Hymenoptera: *Bombus* and *Psithyrus*). Agriculture, Ecosystems & Environment 89: 53-68.

Bakker,J.P. 1989. Nature management by grazing and cutting. On the ecological significance of grazing and cutting regimes applied to restore former species rich grassland communities. Kluwer Academic Press, Groningen.

Bakker,J.P. & Olff,H. 1992. Restoration management in moist grasslands in the Netherlands. Norddeutsche Naturschutzakademie Berichte 5: 42-48.

Bakker,J.P., Dekker,M. & de Vries,Y. 1980. The effect of different management practices on a grassland community and the resulting fate of seedlings. Acta Botanica Neerlandica 29: 469-482.

Bakker,J.P., Poschlod,P., Strykstra,R.J., Bekker,R.M. & Thompson,K. 1996. Seed banks and seed dispersal: important topics in restoration ecology. Acta Botanica Neerlandica 45: 461-490.

Bascompte,J., Jordano,P., Melián,C.J. & Olesen,J.M. 2003. The nested assembly of plant-animal mutualistic networks. PNAS 100: 9383-9387.

Beattie,A.J. 1972. A technique for the study of insect-born pollen. Pan-Pacific Entomologist 47: 82.

Bekker, R. M. & Kwak, M. M. 2005. Life history traits of plant rarity, with particular reference to hemiparasitic Scrophulariaceae. Folia Geobotanica (in press)

Bekker,R.M., Lammerts,E.-J., Schutter,A. & Grootjans,A.P. 1999. Vegetation development in dune slacks: the role of persistent seed banks. Journal of Vegetation Science 10: 745-754.

Benedek,P. 1997. Structure and diversity of Lucerne pollinating wild bee populations as affected by changing agriculture. Acta Horticulturae 437: 353-357.

Benton,T.G., Vickery,J.A. & Wilson,J.D. 2003. Farmland biodiversity: is habitat heterogeneity the key? Trends in Ecology and Evolution 18: 182-188.

Bignal,E.M. & McCracken,D.I. 1996. Low-intensity farming systems in the conservation of the countryside. Journal of Applied Ecology 33: 413-424.

Bingham,R.A. & Orthner,A.R. 1998. Efficient pollination of alpine plants. Nature 391: 238-239.

Bingham,R.A. & Ranker,T.A. 2000. Genetic diversity in alpine and foothill populations of *Campanula rotundifolia* (Campanulaceae). International Journal of Plant Science 161: 403-411.

Blionis,G.J. & Vokou,D. 2001. Pollination ecology of *Campanula* species on Mt Olympos, Greece. Ecography 24: 287-297.

Blomqvist, M. M. 2005. Restoration of plant species diversity of ditch banks. Ecological constraints and opportunities. Ph.D-thesis, Leiden University, the Netherlands.

Blomqvist,M.M., Bekker,R.M. & Vos,P. 2003a. Restoration of ditch bank plant species richness: the potential of the soil seed bank. Applied Vegetation Science 6: 179-188.

Blomqvist,M.M., Vos,P., Klinkhamer,P.G.L. & ter Keurs,W.J. 2003b. Declining plant species richness of grassland ditch banks- a problem of colonisation or extinction? Biological Conservation 109: 391-406.

Bosch,M. & Waser,N.M. 1999. Effects of local density on pollination and reproduction in *Delphinium nuttallianum* and *Aconitum columbianum* (Ranunculaceae). American Journal of Botany 86: 871-879.

Bronstein,J.L. 1994. Conditional outcomes in mutualistic interactions. Trends in Ecology and Evolution 9: 214-217.

Bronstein,J.L. 1995. The plant-pollinator landscape. In: Hansson,L., Fahrig,L. & Merriam,G. (eds.), Mosaic landscapes and ecological processes, pp. 256-288. Chapman & Hall, London, UK.

Brown,B.J. & Mitchell,R.J. 2001. Competition for pollination: effects of pollen of an invasive plant on seed set of a native congener. Oecologia 129: 43-49.

Brown,B.J., Mitchell,R.J. & Graham,S.A. 2002. Competition for pollination between an invasive species (Purple loosestrife) and a native congener. Ecology 83: 2328-2336.

Broyer,J. & LauransonBroyer,J. 1996. The reproduction of plant species in flooded meadows of the Saone Valley (France) [in French]. Acta Botanica Gallica 143: 353-365.

Brugge,B., van der Spek,E. & Kwak,M.M. 1998. Honingbijen in natuurgebieden? De Levende Natuur 99: 71-76.

Buchmann,S.L. & Nabhan,G.P. 1996. The forgotten pollinators. Island Press, Washington D.C., USA.

Bühler,C. & Schmid,B. 2001. The influence of management regime and altitude on the population structure of Succisa pratensis: implications for vegetation monitoring. Journal of Applied Ecology 38: 689-698.

Bulten,M.C. & Kwak,M.M. 2002. Nature management and insects. Entomologische Berichten 62: 24-26.

Calabuig, I. 2000. Solitary bees and bumblebees in a Danish agricultural landscape. Ph.D-thesis, University of Copenhagen, Denmark.

Campbell,D.R. 1985a. Pollen and gene dispersal: the influences of competition for pollination. Evolution 39: 418-431.

Campbell,D.R. 1985b. Pollinator sharing and seed set of *Stellaria pubera*: competition for pollination. Ecology 66: 544-553.

Campbell,D.R. & Motten,A.F. 1985. The mechanism of competition for pollination between two forest herbs. Ecology 66: 554-563.

Campbell,D.R., Waser,N.M. & Pederson,G.T. 2002. Predicting patterns of mating and potential hybridization from pollinator behavior. The American Naturalist 159: 438-450.

Cane,J.H. 2001. Habitat fragmentation and native bees: a premature verdict? Conservation Ecology 5: [online].

Cane,J.H. & Payne,J.A. 1988. Foraging ecology of the bee *Habropoda laboriosa*, an oligolege of blueberries in the southeastern United States. Annals of the Entomological Society of America 81: 419-427.

Cane,J.H. & Tepedino,V.J. 2001. Causes and extent of declines among native North American invertebrate pollinators: detection, evidence, and consequences. Conservation Ecology 5.

CBS (Centraal Bureau voor de Statistiek), Milieu- en Natuurplanbureau (MNP) & Wageningen Universiteit en Researchcentrum (WUR) 2004. Het Natuurcompendium. KNNV Uitgeverij, Utrecht, the Netherlands.

Celary,W. 2004. A comparative study on the biology of *Macropis fulvipes* (Fabricius, 1804) and *Macropis europaea* Warncke, 1973 (Hymenoptera: Apoidea: Mellitidae). Folia Biologica (Kraków) 52: 81-85.

Chinery,M. 1998. Tirion Nieuwe Insektengids. BV Uitgeversmaatschappij Tirion, Baarn, The Netherlands.

Chittka,L., Gumbert,A. & Kunze,J. 1997. Foraging dynamics of bumble bees: correlates of movements within and between plant species. Behavioral Ecology 8: 239-249.

Chust,G., Pretus,J.Ll., Ducrot,D., Bedòs,A. & Deharveng,L. 2003. Identification of landscape units from an insect perspective. Ecography 26: 257-268.

Chust,G., Pretus,J.Ll., Ducrot,D. & Ventura,D. 2004. Scale dependency of insect assemblages in response to landscape pattern. Landscape Ecology 19: 41-57.

Collinge,S.K., Prudice,K.L. & Oliver,J.C. 2003. Effects of local habitat characteristics and landscape context on grassland butterfly diversity. Conservation Biology 17: 178-187.

Comba,L., Corbet,S.A., Hunt,L. & Warren,B. 1999. Flowers, nectar and insect visits: evaluating British plant species for pollinator-friendly gardens. Annals of Botany 83: 369-383.

Corbet,S.A. 1997. Role of pollinators in species preservation, conservation, ecosystem stability and genetic diversity. Acta Horticulturae 437: 219-228.

Corbet,S.A. 2000a. Butterfly nectaring flowers: butterfly morphology and flower form. Entomologia Experimentalis et Applicata 96: 289-298.

Corbet,S.A. 2000b. Conserving compartments in pollination webs. Conservation Biology 14: 1229-1231.

Corbet,S.A. 2005. Allotropous, hemitropous and eutropous pollination systems in crops and wild flowers. In: Waser,N.M. & Ollerton,J. (eds.), Specialization and generalization in plant-pollinator interactions, pp. (in press). The University of Chicago Press, Chicago, Illinois [in press].

Cox,P.A. & Elmqvist,T. 2000. Pollinator extinction in the Pacific islands. Conservation Biology 14: 1237-1239.

Dafni,A. 1992. Pollination ecology. A practical approach. Oxford University Press, New York, USA.

Dauber,J., Hirsch,M., Simmering,D., Waldhardt,R., Otte,A. & Wolters,V. 2003. Landscape structures as an indicator of biodiversity: matrix effects on species richness. Agriculture, Ecosystems & Environment 98: 321-329.

Davic,R.D. 2003. Linking keystone species and functional groups: a new operational definition of the keystone species concept. Conservation Ecology 7: [online].

de Buck,N. 1990. Flower visiting and pollination ecology of hoverflies (Diptera, Syrphidae) in particular for Belgium. Koninklijk Belgisch Instituut voor Natuurwetenschappen, Brussel/Bruxelles, Belgium.

de Jong,T.J., Waser,N.M. & Klinkhamer,P.G.L. 1993. Geitonogamy: the neglected side of selfing. Trends in Ecology and Evolution 8: 321-325.

De Leo,G.A. & Levin,S. 1997. The multifaceted aspects of ecosystem integrity. Conservation Ecology 1: [online].

de Ruiter,P.C., Neutel,A.-M. & Moore,J.C. 1995. Energetics, patterns of interaction strengths and stability in real ecosystems. Science 269: 1257-1260.

de Vos,O.C. 1983. *Scrophularia nodosa*, adapted to wasp pollination? Acta Botanica Neerlandica 32: 345.

Dicks,L.V., Corbet,S.A. & Pywell,R.F. 2002. Compartmentilization in plant-insect flower visitor webs. Journal of Animal Ecology 71: 32-43.

Dijkstra,A.C.J., De Vries,J. & Hoentjen,B.J. 2003. Dagvlinders in Drenthe: voorkomen en verspreiding 1990-2001. PubliQue, Emmen, the Netherlands.

Dlussky,G.M. 1998. Mechanisms of competition for pollinators in *Anthriscus sylvestris* Hoffm. and *Aegopodium podagria* L. (Apiaceae) [in Russian]. Zhurnal Obshchei Biologii 59: 24-44.

Donaldson,J., Nänni,I., Zachariades,C. & Kemper,J. 2002. Effects of habitat fragmentation on pollinator diversity and plant reproductive success in Renosterveld Shrublands of South Africa. Conservation Biology 16: 1267-1276.

Dover,J.W. 1996. Factors affecting the distribution of satyrid butterflies on arable farmland. Journal of Applied Ecology 33: 723-734.

Dover,J.W., Sotherton,N. & Gobbett,K. 1990. Reduced pesticide inputs on cereal field margins: the effects on butterfly abundance. Ecological Entomology 15: 17-24.

Dramstad,W.E., Fry,G.L.A. & Schaffer,M.J. 2003. Bumblebee foraging- is closer really better? Agriculture, Ecosystems & Environment 95: 349-357.

Dunne,J.A., Williams,R.J. & Martinez,N.D. 2002. Network structure and biodiversity loss in food webs: rubustness increases with connectance. Ecology Letters 5: 558-567.

Dupont,Y.L., Hansen,D.M. & Olesen,J.M. 2003. Structure of a plant-flower-visitor network in the high-altitude sub-alpine desert of Tenerife, Canary islands. Ecography 26: 301-310.

Dupont,Y.L., Hansen,D.M., Rasmussen,J.T. & Olesen,J.M. 2004. Evolutionary changes in nectar sugar composition associated with switches between bird and insect pollination: the Canarian bird-flower element revisited. Functional Ecology 18: 670-676.

Eckhart,V.M. 1995. Spatio-temporal variation in abundance and variation in foraging behavior of the pollinators of the gynodioecious *Phacelia linaris* (Hydrophyllaceae). Oikos 64: 573-586.

Elberling,H. & Olesen,J.M. 1999. The structure of a high-latitude plant-pollinator system: The dominance of flies. Ecography 22: 314-323.

Ellis,W.N. & Ellis-Adam,A.C. 1993. To make a meadow it takes a clover and a bee: the entomophilous flora of N.W. Europe and its insects. Bijdragen tot de Dierkunde 63: 193-220.

Ellis,W.N. & Ellis-Adam,A.C. 1994. De ene schermbloem is de andere niet. Entomologische Berichten, Amsterdam 54: 191-199.

Elzinga, J. A. 2005. The effects of habitat fragmentation on a tritrophic system: *Silene latifolia, Hadena bicruris* and its parasitoids. Ph.D-thesis, Netherlands Institute of Ecology (Heteren) and University of Utrecht, the Netherlands.

Fægri,K. & van der Pijl,L. 1979. The principles of pollination ecology. Pergamon Press, London, UK.

Fahrig,L. 2003. Effects of habitat fragmentation on biodiversity. Annual Review of Ecology, Evolution, and Systematics 34: 487-515.

Feinsinger,P. 1987. Effects of plant species on each other's pollination: Is community structure influenced? Trends in Ecology and Evolution 2: 123-126.

Feinsinger,P., Murray,K.G., Kinsman,S. & Busby,W.H. 1986. Floral neighbourhood and pollination success in four hummingbird-pollinated cloud forest species. Ecology 67: 449-464.

Feldman,T.S., Morris,W.F. & Wilson,G. 2004. When can two plant species facilitate each other's pollination? Oikos 105: 197-207.

Fenster,C.B. & Dudash,M.R. 2001. Spatiotemporal variation in the role of hummingbirds as pollinators of *Silene virginica*. Ecology 82: 844-851.

Fishbein,M. & Venable,D.L. 1996. Diversity and temporal change in the effective pollinators of *Asclepias tuberosa*. Ecology 77: 1061-1073.

Fisher,B.L. 1998. Insect behavior and ecology in conservation: preserving functional species interactions. Conservation and Biodiversity 91: 156-158.

Forup,M.L. & Memmott,J. 2005. The restoration of plant-pollinator interactions in hay meadows. Restoration Ecology 13: 265-274.

Fussell,M. & Corbet,S.A. 1992. Flower usage by bumble-bees: a basis for forage plant management. Journal of Applied Ecology 29: 451-465.

Gardener,M.C. & Gillman,M.P. 2001. The effects of soil fertilizer on amino acids in the floral nectar of cornockle, *Agrostemma githago* (Caryophyllaceae). Oikos 92: 101-106.

Garlaschelli,D., Caldarelli,G. & Pietronero,L. 2003. Universal scaling relations in food webs. Nature 423: 165-168.

Geertsema,W. 1999. Spatial distribution patterns of plant species in ditch banks. Aspects of Applied Biology 54: 361-366.

Geertsema, W. 2002. Plant survival in dynamic habitat networks in agricultural landscapes. Ph.D-thesis, Wageningen University, the Netherlands.

Geertsema,W. & Sprangers,J.T.C.M. 2002. Plant distribution patterns related to species characteristics and spatial and temporal habitat heterogeneity in a network of ditch banks. Plant Ecology 162: 91-108.

Gerloff,C.U., Ottmer,B.K. & Schmid-Hempel,P. 2003. Effects of inbreeding on immune response and body size in a social insect, *Bombus terrestris*. Functional Ecology 17: 582-589.

Goulson,D. 1994. A model to predict the influence of insect flower constancy on interspecific competition between insect pollinated plants. Journal of Theoretical Biology 168: 309-314.

Goulson,D. 1999. Foraging strategies of insects for gathering nectar and pollen, and implications for plant ecology and evolution. Perspectives in Plant Ecology, Evolution and Systematics 2: 185-209.

Goulson,D. 2003. Effects of introduced bees on native ecosystems. Annual Review of Ecology, Evolution, and Systematics 34: 1-26.

Goulson,D. & Wright,N.P. 1997. Flower constancy in the hoverflies *Episyrphus balteatus* (Degeer) and *Syrphus ribesii* (L.) (Syrphidae). Behavioral Ecology 9: 213-219.

Goulson,D., Ollerton,J. & Sluman,C. 1997a. Foraging strategies in the small skipper butterfly, *Thymelicus flavus*: when to switch? Animal Behaviour 53: 1009-1016.

Goulson,D., Stout,J.C. & Hawson,S.A. 1997b. Can flower constancy in nectaring butterflies be explained by Darwin's interference hypothesis? Oecologia 112: 225-231.

Goulson,D., Hughes,W.O.H., Derwent,L.C. & Stout,J.C. 2001. Colony growth of the bumblebee, *Bombus terrestris*, in improved and conventional agricultural and suburban habitats. Oecologia 130: 267-273.

Goulson,D., Hanley,M.E., Darvill,B., Ellis,J.S. & Knight,M.E. 2005. Causes of rarity in bumblebees. Biological Conservation 122: 8.

Griebeler,E.M. & Seitz,A. 2002. An individual based model for the conservation of the endangered Large Blue Butterfly, Maculinea arion (Lepidoptera: Lycaenidae). Ecological Modelling 156: 43-60.

Groenendijk,D., van Mannekes,M., Vaal,M. & van den Berg,M. 2002. Butterflies and insecticides: a review and risk analysis of modern Dutch practice. Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society (NEV) 13: 29-34.

Grootjans,A.P., Bakker,J.P., Jansen,A.J.M. & Kemmers,R.H. 2002. Restoration of brook valley meadows in the Netherlands. Hydrobiologia 478: 149-170.

Hansson,M.L. & Persson,T.S. 1994. *Anthriscus sylvestris*- a growing conservation problem. Annales Botanici Fennici 31: 205-213.

Harder,L.D., Williams,N.M., Jordan,C.Y. & Nelson,W.A. 2001. The effects of floral design and display on pollinator economics and pollen dispersal. In: Chittka,L. & Thomson,J.D. (eds.), Cognitive ecology of pollination: animal behavior and floral evolution, pp. 297-317. Cambridge University Press, Cambridge, UK.

Haslett,J.R. 1989. Interpreting patterns of resource utilization: randomness and selectivity in pollen feeding by adult hoverflies. Oecologia 78: 433-442.

Heinrich,B. 1979a. Bumblebee economics. Harvard University Press, Cambridge- London, UK.

Heinrich,B. 1979b. 'Majoring' and 'minoring' by foraging bumblebees, *Bombus vagans*: An experimental analysis. Ecology 60: 245-255.

Heithaus,E.R. 1974. The role of plant-pollinator interactions in determining community structure. Annals of the Missouri Botanical Garden 61: 675-691.

Heithaus,E.R. 1979. Flower-feeding specialization in wild bee and wasp communities in seasonal neotropical habitats. Oecologia 42: 179-194.

Herrera,C.M. 1987. Components of pollinator "quality": comparative analysis of a diverse insect assemblage. Oikos 50: 79-90.

Herrera,C.M. 1988. Variations in mutualisms: the spatio-temporal mosaic of a pollinator assemblage. Biological Journal of the Linean Society 35: 95-125.

Herrera,C.M. 1989. Pollinator abundance, morphology, and flower visitation rate: analysis of the "quantity" component in a plant-pollinator system. Oecologia 80: 241-248.

Herrera,C.M. 1995. Microclimate and individual variation in pollinators: Flowering plants are more than their flowers. Ecology 76: 1516-1524.

Herrera,C.M. 1996. Floral traits and plant adaptation to insect pollinators: A devil's advocate approach. In: Lloyd,D.G. & Barrett,S.C.H. (eds.), Floral biology: Studies in animal-pollinated plants, pp. 65-87. Chapman & Hall, New York.

Herrera,C.M. 2000. Flower-to-seedling consequences of different pollination regimes in an insect-pollinated shrub. Ecology 81: 15-29.

Hill,J.K. & Fox,R. 2003. Climate change and British butterfly distributions. Biologist 50: 106-110.

Hill,P.S.M., Hollis,J. & Wells,H. 2001. Foraging decisions in nectarivores: unexpected interactions between flower constancy and energetic rewards. Animal Behaviour 62: 729-737.

Hoffmann,M.H., Bremer,M., Schneider,K., Burger,K., Stolle,E. & Moritz,G. 2003. Flower visitors in a natural population of *Arabidopsis thaliana*. Plant Biology 5: 491-494.

Holland,J. & Fahrig,L. 2000. Effect of woody borders on insect density and diversity in field crops: a landscape-scale analysis. Agriculture, Ecosystems & Environment 78: 115-122.

Hooftman,D.A.P. & Diemer,M. 2002. Effects of small habitat size and isolation on the population structure of common wetland species. Plant Biology 4: 720-728.

Hooftman,D.A.P., van Kleunen,M. & Diemer,M. 2003. Effects of habitat fragmentation on the fitness of two common wetland species, *Carex davalliana*, and *Succisa pratensis*. Oecologia 134: 350-359.

Hooftman,D.A.P., Billeter,R.C., Schmid,B. & Diemer,M. 2004. Genetic effects of habitat fragmentation on common species of Swiss meadows. Conservation Biology 18: 1043-1051.

Hunneman,H., Hoffmann,F. & Kwak,M.M. 2004. The importance of syrphid flies as pollinators of *Succisa pratensis* (Dipsacaceae). Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society (NEV) 15: 53-58.

Jacquemyn,H., Brys,R., Van Rossum,F., Endels,P., Hermy,M., Triest,L. & De Blust,G. 2002. Behoud van zeldzame plantensoorten in kleine landschapselementen: Sleutelbloemen als voorbeeld. Natuur. focus 1: 19-24.

Jennersten,O. 1984. Flower visitation and pollination efficiency of some North European butterflies. Oecologia 63: 80-89.

Jennersten,O. 1988. Pollination in *Dianthus deltoides* (Caryophyllaceae): Effects of habitat fragmentation on visitation and seed set. Conservation Biology 2: 359-366.

Jennersten,O. & Kwak,M.M. 1991. Competition for bumblebee visitation between *Melampyrum pratense* and *Viscaria vulgaris* with healthy and *Ustilago*-infected flowers. Oecologia 86: 88-98.

Johnson,S.D. & Steiner,K.E. 2000. Generalization versus specialization in plant pollination systems. Trends in Ecology and Evolution 15: 140143.

Jordano,P. 1987. Patterns of mutualistic interactions in pollination and seed dispersal: Connectance, dependence asymmetries, and coevolution. The American Naturalist 129: 657-677.

Jordano,P., Bascompte,J. & Olesen,J.M. 2003. Invariant properties in coevolutionary networks of plant-animal interactions. Ecology Letters 6: 69-81.

Kalisz,S. & Vogler,D.W. 2003. Benefits of autonomous selfing under unpredictable pollinator environments. Ecology 84: 2928-2924.

Kandori,I. 2002. Diverse visitors with various pollinator importance and temporal change in the important pollinators of *Geranium thunbergii* (Geraniaceae). Ecological Research 17: 283-294.

Kato,M. 1996. Plant-pollinator interactions in the understory of a lowland mixed diperocarp forest in Sarawak. American Journal of Botany 83: 732-743.

Kearns,C.A. & Inouye,D.W. 1997. Pollinators, flowering plants, and conservation biology. Bioscience 47: 297-307.

Kearns,C.A., Inouye,D.W. & Waser,N.M. 1998. Endangered mutualisms: The conservation of plant-pollinator interactions. Annual Review of Ecology and Systematics 29: 83-112.

Kennedy,T.A., Naeem,S., Howe,K.M., Knops,J.M.H., Tilman,D. & Reich,P.B. 2002. Biodiversity as a barrier to ecological invasion. Nature 417: 636-638.

Kevan,P.G. 1999. Pollinators as bioindicators of the state of the environment: species, activity and diversity. Agriculture, Ecosystems & Environment 74: 373-393.

Keys,R.N., Buchmann,S.L. & Smith,S.E. 2005. Pollination effectiveness and pollination efficiency of insects foraging on *Prosopis velutina* in south-eastern Arizona. Journal of Applied Ecology 32: 519-527.

Kleijn,D. & Sutherland,J.P. 2004. How effective are European agri-environment schemes in conserving and promoting biodiversity? Journal of Applied Ecology 40: 947-969.

Kleijn,D., Joenje,W. & Kropff,M.J. 1997. Patterns in species composition of arable field boundary vegetation. Acta Botanica Neerlandica 46: 175-192.

Kleijn,D., Boekhoff,M., Ottburg,F., Gleichman,M. & Berendse,F. 1999. De effectiviteit van agrarisch natuurbeheer. Evaluatie van natuurwinst door beheersovereenkomsten in de polders Westbroek en Maarsseveen. Landschap 16: 227-235.

Kleijn,D., Berendse,F., Smit,R. & Gilissen,N. 2001. Agri-environment schemes do not effectively protect biodiversity in Dutch agricultural landscapes. Nature 413: 723-725.

Knuth,P. 1898. Handbuch der Blütenbiologie 1-3. W. Engelmann, Leipzig.

Koniuszek,J.W.J., Bast-Cramer,W.B., Geerlings,R.A. & Verkleij,J.A.C. 1986. Estimation of selfing rate in a natural population of *Scrophularia nodosa* L. using allozymes. Acta Botanica Neerlandica 35: 393-403.

Kremen,K. & Ricketts,T. 2000. Global perspectives on pollination disruptions. Conservation Biology 14: 1226-1228.

Kunin,W.E. 1993. Sex and the single mustard: population density and pollinator behavior effects on seed set. Ecology 74: 2145-2160.

Kwak,M.M. 1979. Effects of bumblebee visits on the seed set of *Pedicularis, Rhianthus* and *Melampyrum* (Scrophulariaceae) in the Netherlands. Acta Botanica Neerlandica 28: 177-195.

Kwak,M.M. 1980. The pollination value of honeybees to the bumblebee plant *Rhinanthus*. Acta Botanica Neerlandica 29: 597-603.

Kwak,M.M. 1988. Pollination ecology and seed-set in the rare annual species *Melampyrum arvense* L. (Scrophulariaceae). Acta Botanica Neerlandica 37: 153-163.

Kwak,M.M. 1993. The relative importance of syrphids and bumblebees as pollinators of three plant species. Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society (NEV) 4: 137-143.

Kwak,M.M. 1994a. Planten en bestuivers: achteruitgang leidt tot verschuivende relaties. Landschap 11: 29-39.

Kwak,M.M. 1994b. Populatiestructuur en bestuiving: effecten van ruimtelijke rangschikking bij de Zwartblauwe Rapunzel. Landschap 11: 15-24.

Kwak,M.M. & Jennersten,O. 1986. The significance of pollination time and frequency and purity of pollen loads for seed set in *Rhinanthus angustifolius* (Scrophulariaceae) and *Viscaria vulgaris* (Caryophyllaceae). Oecologia 70: 502-507.

Kwak,M.M. & Jennersten,O. 1991. Bumblebee visitation and seedset in *Melampyrum pratense* and *Viscaria vulgaris*: heterospecific pollen and pollen limitation. Oecologia 86: 99-104.

Kwak,M.M. & Bergman,P. 1996. Early flowers of *Bartsia alpina* (Scrophulariaceae) and the visitation by bumblebees. Acta Botanica Neerlandica 45: 355-366.

Kwak,M.M. & Vervoort,N. 2000. Bumblebee visitation and dispersal of fluorescent dye powder between bridges and isolated experimental patches of *Phyteuma nigrum* (Campanulaceae). Det Norske Videnskaps Akademi. I. Matematisk-Naturvidenskapelige Klasse, Skrifter, Ny Serie 39: 11-21.

Kwak,M.M. & Bekker,R.M. 2005. Ecology of plant reproduction: extinction risks and restoration perspectives of rare plant species. In: Waser,N.M. & Ollerton,J. (eds.), Specialization and generalization in plant-pollinator interactions, pp. (in press). The University of Chicago Press, Chicago, Illinois, USA [in press].

Kwak,M.M., Velterop,O. & Boerrigter,E.J.M. 1996. Insect diversity and the pollination of rare plant species. In: Matheson,A., Buchmann,S.L., O'Toole,C., Westrich,P. & Williams,I.H. (eds.), The conservation of bees, pp. 115-124. Academic Press, London.

Kwak,M.M., Velterop,O. & van Andel,J. 1998. Pollen and gene flow in fragmented habitats. Applied Vegetation Science 1: 37-54.

Laverty,T.M. 1991. Plant interactions for pollinator visits: a test of the magnet species effect. Oecologia 89: 502-508.

Le Cœur,D., Baudry,J., Burel,F. & Thenail,C. 2002. Why and how we should study field boundary biodiversity in an agrarian landscape context. Agriculture, Ecosystems & Environment 89: 23-40.

Lehman,C.L. & Tilman,D. 2000. Biodiversity, stability and productivity in competitive communities. The American Naturalist 156: 534-552.

Lindborg,R. & Erikson,O. 2004. Historical landscape connectivity affects present plant species diversity. Ecology 85: 1840-1845.

Loew,E. 1895. Einführung in die Blütenbiologie auf historischer Grundlage. Dummlers Verlagsbuchhandlung, Berlin, Germany.

Londo,G. 1974. Over de najaarsbloei van graslandplanten. De Levende Natuur 77: 44-48.

Loreau,M., Naeem,S. & Inchausti,P. 2002. Biodiversity and ecosystem functioning. Oxford University Press, New York, USA.

Luijten, S. H. 2001. Reproduction and genetics of fragmented plant populations. Ph.D-thesis, University of Amsterdam, the Netherlands.

Luijten,S.H., Dierick,A., Oostermeijer,J.G.B., Raijman,L.E.L. & Den Nijs,H.C.M. 2000. Population size, genetic variation, and reproductive success in a rapidly declining, self-incompatible perennial (*Arnica montana*) in The Netherlands. Conservation Biology 14: 1776-1787.

Magurran,A.E. 1988. Ecological diversity and its measurement. Croom Helm Ltd., London, United Kingdom.

Mänd,M., Mänd,R. & Williams,I.H. 2002. Bumblebees in the agricultural landscape of Estonia. Agriculture, Ecosystems & Environment 89: 69-76.

Manhoudt,A.G.E. & de Snoo,G.R. 2003. A quantitative survey of semi-natural habitats on Dutch arable farms. Agriculture, Ecosystems & Environment 97: 235-240.

Mayfield,M.M., Waser,N.M. & Price,M.V. 2001. Exploring the 'most effective pollinator principle' with complex flowers: bumblebees and *Ipomopsis aggregata*. Annals of Botany 88: 591-596.

McCann,K.S. 2000. The diversity-stability debate. Nature 405: 228-233.

Memmott,J. 1999. The structure of a plant-pollinator food web. Ecology Letters 2: 276-280.

Memmott,J., Martinez,N.D. & Cohen,J.E. 2000. Predators, parasitoids and pathogens: species richness, trophic generality and body sizes in a natural food web. Journal of Animal Ecology 69: 1-15.

Memmott,J., Waser,N.M. & Price,M.V. 2004. Tolerance of pollination networks to species extinctions. Proc. R. Soc. Lond. B 271: 2605-2611.

Mills,L.S., Soulé,M.E. & Doak,D.F. 1993. The keystonespecies-concept in ecology and conservation. Bioscience 43: 219-224.

Mitchell,R.J. 1993. Path analysis: Pollination. In: Scheiner,S.M. & Gurevitch,J. (eds.), Design and analysis of ecological experiments, pp. 211-231. Chapman & Hall, New York, USA.

Moldenke,A.R. 1975. Niche specialization and species diversity along a California Transect. Oecologia 21: 219-242.

Murren,C.J. 2002. Effects of habitat fragmentation on pollination: pollinators, pollinia viability and reproductive success. Journal of Ecology 90: 100-107.

Mustajärvi,K., Siikamäki,P., Rytkönen,S. & Lammi,A. 2001. Consequences of plant population size and density for plant-pollinator interactions and plant performance. Journal of Ecology 89: 80-87.

Nakano,C. & Washitani,I. 2003. Variability and specialisation of plant-pollinator systems in a northern maritime grassland. Ecological Research 18: 221-246.

Nathan,R. & Muller-Landau,H.C. 2000. Spatial patterns of seed dispersal, their determinants and consequences for recruitment. Trends in Ecology and Evolution 15: 278-285.

Neal,P.R. 1998. Pollinator restoration. Trends in Ecology and Evolution 13: 132-133.

Neff,J.L. & Simpson,B.B. 1993. Bees, pollination systems and plant diversity. In: Lasalle,J. & Gauld,J.D. (eds.), Hymenoptera and biodiversity, pp. 143-167. CAB International, Wallingford, UK.

NyHagen,D.F., Kragelund,C., Olesen,J.M. & Jones,C.G. 2001. Insular interactions between lizards and flowers: flower visitation by an endemic Mauritian gecko. Journal of Tropical Ecology 17: 755-761.

Nyman,Y. 1992. Pollination mechanisms in six *Campanula* species (Campanulaceae). Plant Systematics and Evolution 181: 97-108.

Olesen,J.M. 2000. Exactly how generalised are pollination interactions? Det Norske Videnskaps Akademi. I. Matematisk-Naturvidenskapelige Klasse, Skrifter, Ny Serie 39: 161-178.

Olesen,J.M. & Jain,S.K. 1994. Fragmented populations and their lost interactions. In: Loeschke,V., Tomiuk,J. & Jain,S.K. (eds.), Conservation genetics, pp. 417-426. Birkhauser Verlag, Basel.

Olesen,J.M. & Jordano,P. 2002. Geographic patterns in plant-pollinator mutualistic networks. Ecology 83: 2416-2424.

Olesen,J.M., Esklidsen,L.I. & Venkatasamy,S. 2002. Invasion of pollination networks on oceanic islands: importance of invader complexes and endemic super generalists. Diversity and Distributions 8: 181-192.

Ollerton,J. & Cranmer,L. 2002. Latitudinal trends in plant-pollinator interactions: are tropical plants more specialised? Oikos 98: 340-350.

Ollerton,J. & Watts,S. 2000. Phenotype space and floral typology: Towards an objective assessment of pollination syndromes. Det Norske Videnskaps Akademi. I. Matematisk-Naturvidenskapelige Klasse, Skrifter, Ny Serie 149-159.

Olsen,K.M. 1997. Pollination effectiveness and pollinator importance in a population of *Heterotheca subaxxillaris* (Asteraceae). Oecologia 109: 114-121.

Oostermeijer,J.G.B., Den Nijs,H.C.M., Raijman,L.E.L. & Menken,S.B.J. 1992. Population biology and management of the Marsh gentian *Gentiana pneumonanthe* L., a rare species in the Netherlands. Botanical Journal of the Linean Society 108: 117-129.

Oostermeijer,J.G.B., van Eijck,M.W. & den Nijs,J.C.M. 1994. Offspring fitness in relation to population size and genetic variation in the rare perennial plant species *Gentiana pneumonanthe* (Gentianaceae). Oecologia 97: 289-296.

Oostermeijer,J.G.B., Luijten,S.H., Petanidou,T., Kos,M., Ellis-Adam,A.C. & Den Nijs,H.C.M. 2000. Pollination in rare plants: is population size important? Det Norske Videnskaps Akademi. I. Matematisk-Naturvidenskapelige Klasse, Skrifter, Ny Serie 39: 201-213.

Osborne,J.L. & Corbet,S.A. 1994. Managing habitats for pollinators in farmland. Aspects of Applied Biology 40: 207-215.

Osborne,J.L. & Williams,I.H. 2001. Site constancy of bumble bees in an experimentally patchy habitat. Agriculture, Ecosystems & Environment 83: 129-141.

Osborne,J.L., Clark,S.J., Morris,R.J., Williams,I.H., Riley,J.R., Smith,A.D., Reynolds,D.R. & Edwards,A.S. 1999. A landscape-scale study of bumble bee foraging range and constancy, using harmonic radar. Journal of Applied Ecology 36: 519-533.

Ottenheim,M.M. 2000. Annual and diurnal rhythms of *Eristalis* species (Diptera: Syrphidae). Procedings of Experimental and Applied Entomology 11: 169-175.

Ouborg,N.J., van Treuren,R. & van Damme,J.M.M. 1991. The significance of genetic erosion in the process of extinction. II. Morphological variatiion and fitness components in populatioons of variying size of *Salvia pratensis* L. and *Scabiosa columbaria* L. Oecologia 86: 359-367.

Owen,O.T., Memmott,J., Lasalle,J., Lyal,C.H.C., Whitefoord,C. & Godfray,H.C.J. 2002. Structure of a diverse tropical forest parasitoid community. Journal of Animal Ecology 71: 855-873.

Paine,R.T. 1969. A note on trophic complexity and community stability.The American Naturalist 103:91-93.

Paton,D.C. 2000. Disruption of bird-plant pollination systems in Southern Australia. Conservation Biology 14: 1232-1234.

Peeters,T.M.J. & Reemer,M. 2003. Bedreigde en verdwenen bijen in Nederland (Apidae s.l.). Basisrapport met voorstel voor de Rode Lijst. Stichting European Invertebrate Survey- Nederland, Leiden, the Netherlands.

Peeters,T.M.J., Raemakers,I.P. & Smit,J. 1999. Voorlopige atlas van de Nederlandse bijen (Apidae). European Invertebrate Survey- Nederland, Leiden.

Peeters,T.M.J., van Achterberg,C., Heitmans,W.R.B., Klein,W.F., Lefeber,V., van Loon,A.J., Mabelis,A.A., Nieuwenhuijsen,H., Reemer,M., de Rond,J., Smit,J. & Velthuis,H.H.W. 2004. De wespen en mieren van Nederland (Hymenoptera: Aculeata). Nationaal Natuurhistorisch Museum Naturalis, KNNV Uitgeverij and European Invertebrate Survey- Nederland, Leiden, the Netherlands.

Pekkarinen,A., Berg,Ø., Calabuig,I., Janzon,L.-Å. & Luig,J. 2003. Distribution and co-existance of the *Macropis* species and their cleptoparasite *Epeoloides coecutiens* (Fabr.) in NW Europe (Hymenoptera: Apoidea, Melittidae and Apidae). Entomologica Fennica 14: 53-59.

Petanidou, T. 1991. Pollination ecology in a phryganic ecosystem. Ph.D-thesis, Aristotelian University, Thessaloniki, Greece.

Petchey,O.L. 2004. On the statistical significance of functional diversity effects. Functional Ecology 18: 297-303.

Pettersson,M.W. 1991. Pollination by a guild of fluctuating moth populations: option for unspecialization the Bladder in Silene vulgaris. Journal of Ecology 79: 591-604.

Plate,C., Liefaard,R. & van Duuren,L. 1992. Veranderingen in de Nederlandse flora op basis van de Standaardlijst 1990. Gorteria 18: 21-29.

Potts,S.G., Vulliamy,B., Dafni,A., Ne'eman,G. & Willmer,P. 2003. Linking bees and flowers: how do floral communities structure pollinator communities? Ecology 84: 2628-2642.

Power,M.E., Tilman,D., Estes,J.A., Menge,B.A., Bond,W.J., Mills,L.S., Daily,G., Castilia,J.C. & Paine,R.T. 1996. Challenges in the quest for keystones. Bioscience 46: 609-620.

Primack,R.B. 1985. Longevity of individual flowers. Annual Review of Ecology, Evolution, and Systematics 16: 15-37.

Proctor,M.C.F. 1978. Insect pollination syndromes in an evolutionary and ecosystem context. In: Richards,A.J. (ed.), The pollination of flowers by insects, The Linnean Society Symposium Series 6, pp. 105-116. The Linnean Society of London, Academic Press, London, UK.

Proctor,M. & Yeo,P. 1973. The pollination of flowers. Collins, London, UK.

Proctor,M., Yeo,P. & Lack,A. 1996. The natural history of pollination. Harper Collins Publishers, London, UK.

Quinn,R.M., Lawton,J.H., Eversham,B.C. & Wood,S.N. 1994. The biogeography of scarce vascular plants in Britain with respect to habitat preference, dispersal ability and reproductive biology. Biological Conservation 70: 149-157.

Raemakers,I.P., Schaffers,A.P., S_kora,K.V. & Heijerman,T. 2001. The importance of plant communities in road verges as a habitat for insects. Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society (NEV) 12: 101-106.

Ramsey,M. 1995. Causes and consequences of seasonal variation in pollen limitation and seed production in *Blandfordia grandiflora* (Liliaceae). Oikos 73: 49-58.

Randall,J.L. & Hilu,K.W. 1990. Interference through improper pollen transfer in mied stands of *Impatiens capensis* and *I. pallida* (Balsaminaceae). American Journal of Botany 77: 939-944.

Rasmont, P. 1988. Monographie écologique et zoogéographique des bourdons de France et de Belgique (Hymenoptera, Apidae, Bombinae). Ph.D-thesis, Faculté universitaire des Sciences agronomique de l'État, Gembloux, Belgium.

Rasmussen,C. & Olesen,J.M. 2000. Oil flowers and oil-collecting bees. Det Norske Videnskaps Akademi. I. Matematisk-Naturvidenskapelige Klasse, Skrifter, Ny Serie 39: 23-31.

Rathcke,B.J. 1983. Competition and facilitation among plants for pollination. In: Real,L. (ed.), Pollination Biology, pp. 305-329. Academic Press, Orlando, Florida, USA.

Rathcke,B.J. & Jules,E.S. 1993. Habitat fragmentation and plant-pollinator interactions. Current Science 65: 273-277.

Richards,A.J. 2001. Does low biodiversity resulting from modern agriculture practice affect crop pollination and yield? Annals of Botany 88: 165-172.

Roubik,D.W. 2000. Pollination system stability in tropical America. Conservation Biology 14: 1237-1239. Salveter,R. 1998a. Habitatnutzung adulter Schwebfliegen (Diptera: Syrphidae) in einer stark gegliederten Agrarlandschaft. Mitteilungen der Schweizerischen Entomologischen Gesellschaft 71: 49-71.

Salveter,R. 1998b. The influence of sown herb strips and spontaneous weeds on the larval stages of aphidophagous hoverflies (Dipt., Syrphidae). Journal of Applied Entomology 122: 103-114.

Samways,M.J. 2005. Insect diversity conservation. Cambridge University Press, Cambridge, UK.

Saville,N.M., Dramstad,W.E., Fry,G.L.A. & Corbet,S.A. 1997. Bumblebee movement in a fragmented agricultural landscape. Agriculture, Ecosystems & Environment 61: 145-154.

Schaffers, A. P. 2000. Ecology of roadside plant communities. Ph.Dthesis, Wageningen University, the Netherlands.

Schaffers,A.P. 2002. Soil, biomass, and management of semi-natural vegetation. Part II. Factors controlling species diversity. Plant Ecology 158: 268.

Schaffers,A.P., Vesseur,M.C. & Sýkora,K.V. 1998. Effects of delayed hay removal on the nutrient balance of roadside plant communities. Journal of Applied Ecology 35: 349-364.

Schemske,D.W. 1981. Floral convergence and pollinator sharing in two bee-pollinated tropical herbs. Ecology 62: 946-954.

Schemske,D.W. & Horvitz,C.C. 1989. Temporal variation in selection on a floral character. Evolution 43: 461-465.

Schoenly,K. & Cohen,J.E. 1991. Temporal variation in food web structure: 16 emperical classes. Ecological Monographs 61: 267-298.

Schulke,B. & Waser,N.M. 2001. Long-distance pollinator flights and pollen dispersal between populations of Delphinium nuttallianum. Oecologia 127: 239-245.

Shipley,B. 1997. Exploratory path analysis with applications in ecology and evolution. The American Naturalist 149: 1113-1138.

Sih,A. & Baltus,M.S. 1987. Island size, pollinator behavior and pollination limitation in catnip. Ecology 68: 1679-1690.

Skov,C. 2000. Oligolectic bees in Denmark: How and why are they specialised in their foraging? Det Norske Videnskaps Akademi. I. Matematisk-Naturvidenskapelige Klasse, Skrifter, Ny Serie 39: 43-53.

Slaa,E.J. & Biesmeijer,J.C. 2003. Flower constancy: A review of definitions, measurements and data analysis. In: Dafni,A. & Kevan,P.G. (eds.), Manual of Field Methods in pollination Ecology Enviroquest, Cambridge, Ontario, Canada.

Smithson,A. 2001. Pollinator preference, frequency dependence, and floral evolution. In: Chittka,L. & Thomson,J.D. (eds.), Cognitive ecology of pollination: animal behavior and floral evolution, pp. 237-258. Cambridge University Press, Cambridge, UK.

Sokal,R.R. & Rohlf,F.J. 2001. Biometry. The principles and practice of statistics in biological research. W.H. Freeman and Company, New York, USA.

Soons,M.B. 2002. Reduced colonization capacity in fragmented populations of wind-dispersed grassland forbs. Journal of Ecology 90: 1033-1043.

Soons, M. B. 2003. Habitat fragmentation and connectivity. Spatial and temporal characteristics of the colonization process in plants. Ph.D-thesis, Utrecht University, the Netherlands.

Sowig,P. 1989. Effects of a flowering plant's patch size on species composition of pollinator communities, foraging strategies, and resource partitioning in bumblebees (Hymenoptera: Apidae). Oecologia 78: 550-558.

Speight,M.C.D., Castella,E., Obrdlik,P. & Ball,S.G. 2001. Syrph the Net on CD Issue 1. The database of European Syrphidae. Syrph the Net Publications, Dublin, Rep. of Ireland.

Spek,T. 2004. Het Drentse esdorpenlandschap. Een historisch-geografische studie. Uitgeverij Matrijs, Utrecht, the Netherlands.

Spira,T.P. 2001. Plant-pollinators interactions: a threatened mutualism with implications for ecology and management of rare plants. Natural Areas Journal 21: 78-88.

Stang, M., Klinkhamer, P. G. L. & van der Meijden, E. 2005. Size constraints and flower abundance determine the number of interactions in a plant-flower visitor web. Oikos [in press].

Stefanescu,C., Herrando,S. & Páramo,F. 2004. Butterfly species richness in the north-west Mediterranean Basin: the role of natural and human induced factors. Journal of Biogeography 31: 905-915.

Steffan-Dewenter,I. & Tscharntke,T. 1997. Bee diversity and seed set in fragmented habitats. Acta Horticulturae 437: 231-234.

Steffan-Dewenter,I. & Tscharntke,T. 1999. Effects of habitat isolation on pollinator communities and seed set. Oecologia 121: 432-440.

Stichting Werkgroep Florakartering Drenthe 1999. Atlas van de Drentse flora. Schuyt & Co, Haarlem, the Netherlands.

Strickler,K. 1979. Specialization and foraging efficiency of solitary bees. Ecology 60: 998-1009.

Strykstra,R.J., Verweij,G.L. & Bakker,J.P. 1997. Seed dispersal by mowing machinery in a Dutch brook valley system. Acta Botanica Neerlandica 46: 387-401.

Stubbs,A.E. & Falk,S.J. 1996. British Hoverflies. An illustrated identification guide. British Entomological and Natural History Society, Reading, UK.

Sugden,E.A. 1986. Anthecology and pollinator efficicacy of *Styrax officinalis* subsp. *redivivum* (Styraceae). American Journal of Botany 73: 919-930.

Sutherland,J.P., Sullivan,M.S. & Poppy,G.M. 1999. The influence of floral character on the foraging behaviour of the hoverfly, *Episyrphus balteatus*. Entomologia Experimentalis et Applicata 93: 157-164.

Sutherland,J.P., Sullivan,M.S. & Poppy,G.M. 2001. Distribution and abundance of aphidophagous hoverflies (Diptera: Syrphidae) in wildflower patches and field margins. Agricultural and Forest Entomology 3: 57-64.

Tamis,W.L.M., van der Poll,R.J., de Snoo,G.R. & Donner,J.H. 2001. First results on the effects of different types of unsprayed grass margins on the biodiversity of entomofauna. Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society (NEV) 12: 107-112.

Tamis,W.L.M., van der Meijden,R., Runhaar,J., Bekker,R.M., Ozinga,W.A., Odé,B. & Hoste,I. 2004. Standard list of the flora of the Netherlands 2003. Gorteria 30: 101-195.

Tepedino,V.J., Sipes,S.D., Barnes,J.L. & Hickerson,L.L. 1997. The need for "extended care" in conservation: examples from studies of rare plants in the western United States. Acta Horticulturae 437: 245-248.

ter Borg, S. J. 1972. Variability of *Rhinanthus serotinus* in relation to environment. Ph.D-thesis, University of Groningen, the Netherlands.

Thomas,J.A., Telfer,M.G., Roy,D.B., Preston,C.D., Greenwood,J.J.D., Asher,J., Fox,R., Clarke,R.T. & Lawton,J.H. 2004. Comparative losses of British butterflies, birds, and plants and the global extinction crisis. Science 303: 1879-1881.

Thomson,J.D. 1978. Effects of stand composition on insect visitation in two-species mixtures of *Hieracium*. American Midland Naturalist 100: 413-440.

Thomson,J.D. 1981. Field measures of flower constancy in bumblebees. The American Midland Naturalist 105: 377-380.

Thomson,J.D. 2001. How do visitation patterns vary among pollinators in relation to floral display and floral design in a generalist pollination system? Oecologia 126: 386-394.

Thomson,J.D. & Chittka,L. 2001. Pollinator individuality: when does it matter? In: Chittka,L. & Thomson,J.D. (eds.), Cognitive ecology of pollination: animal behavior and floral evolution, pp. 191-213. Cambridge University Press, Cambridge.

Thomson,J.D. & Goodell,K. 2001. Pollen removal and deposition by honeybee and bumblebee visitors to apple and almond flowers. Journal of Applied Ecology 38: 1032-1044.

Thomson,J.D., Slatkin,M. & Thomson,B.A. 1997. Trapline foraging by bumble bees: II. Definition and detection from sequence data. Behavioral Ecology 8: 199-210.

Thompson,K., Bakker,J.P. & Bekker,R.M. 1997. The soil seed banks of North West Europe: methodology, density and longevity. Cambridge University press, Cambridge, UK.

Tilman,D., Reich,P.B., Knops,J.M.H., Wedin,D., Mielke,T. & Lehman,C. 2001. Diversity and productivity in a long-term grassland experiment. Science 294: 843-845.

Tomimatsu,H. & Ohara,M. 2002. Effects of forest fragmentation on seed production of the understory herb *Trillium camschatcense*. Conservation Biology 16: 1277-1285.

Torres,C. & Galetto,L. 2002. Are nectar sugar composition and corolla tube length related to the diversity of insects that visit Asteraceae flowers? Plant Biology 4: 360-366.

Totland,Ø., Eide,W. & Grytnes,J.A. 2000. Is there a typical alpine flower? Det Norske Videnskaps Akademi. I. Matematisk-Naturvidenskapelige Klasse, Skrifter, Ny Serie 39: 139-148.

Traveset,A. & Sáez,E. 1997. Pollination of *Euphorbia dendroides* by lizards and insects: Spatio-temporal variation in patterns of flower visitation. Oecologia 111: 241-248.

UNEP 1995. Global Biodiversity Assessment. Cambridge University Press, Cambridge, UK.

UNEP 2005. Millennium Ecosystem Assessment Synthesis Report. Pre-publication Final Draft Approved by MA Board on March 23, 2005. Millennium Ecosystem Assessment, UNEP (United Nations Environmental Programme).

van der Goot,V.S. 1981. De zweefvliegen van Noordwest-Europa en EuropeesRusland, in het bijzonder van de Benelux. K.N.N.V., Amsterdam, the Netherlands.

van der Heiden,S.M., Feenstra,H. & Straathof,N. 2005. Hoogveenherstelproject Fochteloërveen goed voor waterhuishouding en fauna? De Levende Natuur 106: 94-101.

van der Meijden,R., Weeda,E.J., Holverda,W.J. & Hovenkamp,P.H. 1996. Heukels' Flora van Nederland, 22e druk. Wolters-Noordhoff, Groningen, the Netherlands.

van der Meijden,R., Odé,B., Groen,C.L.G., Witte,J.-P.M. & Bal,D. 2000. Bedreigde en kwetsbare vaatplanten in Nederland. Basisrapport met voorstel voor de Rode Lijst. Gorteria 26: 85-208.

van der Muren,C., Hoffmann,F. & Kwak,M.M. 2003. Insect diversity on yellow Asteraceae in road verges in the Netherlands. Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society (NEV) 14: 115-118.

van Duren,I.C. & van Andel,J. 1997. Nutrient deficiency in undisturbed, drained and rewetted peat soils tested with Holcus lanatus. Acta Botanica Neerlandica 46: 377-386.

van Duren,I.C., Pegtel,D.M., Aerts,B.A. & Inberg,J.A. 1997. Nutrient supply in undrained and drained *Calthion* meadows. Journal of Vegetation Science 8: 829-838.

van Mierlo,J.E.M. & van Groenendael,J.M. 1991. A population dynamic approach to the control of *Anthriscus sylvestris* (L.) Hoffm. Journal of Applied Ecology 28: 128-139.

van Rossum,F., Echgadda,G., Szabadi,I. & Triest,L. 2002. Commonness and long-term survival in fragmented habitats: *Primula eliatior* as a study case. Conservation Biology 16: 1286-1295.

van Swaay,C.A.M. & Warren,M. 2001. Implementing the *Red Data Book of European Butterflies*: the identification of Prime Butterfly Areas. Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society (NEV) 12: 129-134.

van Treuren,R., Bijlsma,R., Ouborg,N.J. & van Delden,W. 1993. The significance of gentic erosion in the process of extinction. IV. Inbreeding depression and heterosis effects by selfing and outcrossing in *Scabiosa columbaria*. Evolution 47: 1669-1680.

van Treuren,R., Bijlsma,R., Ouborg,N.J. & Kwak,M.M. 1994. Relationships between plant density, outcrossing rates and seed set in natural and experimental populations of Scabiosa columbaria. Journal of Evolutionary Biology 7: 287-302.

Vázquez,D.P. & Simberloff,D. 2002. Ecological specialization and susceptibility to disturbance: conjectures and refutations. The American Naturalist 159: 606-623.

Vázquez,D.P. & Aizen,M.A. 2003. Null model analyses of specialization in plant-pollinator interactions. Ecology 84: 2493-2501.

Velterop, O. 2000. Effects of fragmentation on pollen and gene flow in insect pollinated plant populations. Ph.D-thesis, University of Groningen, the Netherlands.

Vepsäläinen,K. & Savolainen,R. 2000. Are spring mass migrations of bumblebees and wasps driven by vole cyclicity? Oikos 91: 401-404.

Verberk,W.C.E.P., Brock,A.M.T., van Duinen,G.-J., van Es,M., Kuper,J., Peeters,T.M.J., Smits,M.J.A., Timan,L. & Esselink,H. 2002. Seasonal and spatial patterns in macro-invertebrate assemblages in a heterogeneous landscape. Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society (NEV) 13: 35-43.

Vergeer,P., Rengelink,R., Copal,A. & Ouborg,N.J. 2003a. The interacting effects of genetic variation, habitat quality and population size on performance of *Succisa pratensis*. Journal of Ecology 91: 18-26.

Vergeer,P., Rengelink,R., Ouborg,N.J. & Roelofs,J.G.M. 2003b. Effects of population size and genetic variation size on the response of *Succisa pratensis* to eutrophication and acidification. Journal of Ecology 91: 600-609.

Verlaar,H.J. 1990. Corridors as a tool for plant species conservation. In: Bunce,R.G.H. & Howard,D.C. (eds.), Species dispersal in agricultural habitats, pp. 82-97. Belhaven Press, London, UK.

Verlinden,L. 1991. Zweefvliegen. Koninklijk Belgisch Instituut voor Natuurwetenschappen/ Institut Royal des Sciences Naturelles de Belgique, Brussel-Bruxelles, Belgium.

Vessby,K., Söderström,B., Glimskär,A. & Svensson,B. 2002. Species richness of six different taxa in Swedish seminatural grasslands. Conservation Biology 16: 430-439.

Vogel,S. 1976. *Lysimachia*: Ölblumen der Holarktis. Naturwissenschaften 63: 44-45.

Vogel,S. 1986. Ölblumen und ölsammelnde Bienen. Folge 2. *Lysimachia* und *Macropis*. Akad. d. Wiss. u.d. Literatur- Mainz, Steiner-Verlag-Wiesbaden GmbH, Stuttgart, Germany.

Waddington,K.D. 2001. Subjective evaluation and choice behavior by nectar- and pollen-collecting bees. In: Chittka,L. & Thomson,J.D. (eds.), Cognitive ecology of pollination: animal behavior and floral evolution, pp. 41-60. Cambridge University Press, Cambridge, UK.

Walther-Hellwig,K. & Frankl,R. 2000. Foraging habitats and foraging distances of bumblebees, *Bombus* spp. (Hym., Apidae), in an agricultural landscape. Journal of Applied Entomology 124: 299-306.

Waser,N.M. 1978. Interspecific pollen transfer and competition between co-occurring plant species. Oecologia 36: 236.

Waser,N.M. 1979. Effective mutualism between sequentially flowering plant species. Nature 281: 670-672.

Waser,N.M. 1986. Flower constancy: definition, cause and measurement. The American Naturalist 127: 593-603.

Waser,N.M. 2001. Pollinator behavior and plant speciation: looking beyond the "ethological isolation" paradigm. In: Chittka,L. & Thomson,J.D. (eds.), Cognitive ecology of pollination: animal behavior and floral evolution, pp. 318-335. Cambridge University Press, Cambridge, UK.

Waser,N.M. & Fugate,M.L. 1986. Pollen precendence and stigma closure: a mechanism of competition between *Delphinium nelsonii* and *Ipomopsis aggregata*. Oecologia 70: 573-577.

Waser,N.M. & Price,M.V. 1990. Pollination efficiency and effectiveness of bumblebees and hummingbirds visiting *Delphinium nelsonii*. Collecteana Botanica 19: 9-20.

Waser,N.M., Chittka,L., Price,M.V., Williams,N.M. & Ollerton,J. 1996. Generalization in pollination systems, and why it matters. Ecology 77: 1043-1060.

Washitani,I. 1996. Predicted genetic consequences of strong fertility selection due to pollinator loss in an isolated population of *Primula sieboldii*. Conservation Biology 10: 59-64.

Weeda,E.J., Westra,R., Westra,C. & Westra,T. 1985. Nederlandse ecologische flora. Wilde planten en hun relaties. KNNV Uitgeverij/ IVN, Hilversum, the Netherlands.

Weiss,M.R. 2001. Vision and learning in some neglected pollinators: beetles, flies, moths, and butterflies. In: Chittka,L. & Thomson,J.D. (eds.), Cognitive ecology of pollination: animal behavior and floral evolution, pp. 171-190. Cambridge University Press, Cambridge, UK.

Weller,S.G. 1995. The relationship of rarity to plant reproductive biology. In: Bowles,M.L. & Whelan,C.J. (eds.), Restoration of endangered species: conceptual issues, planning, and implementation., pp. 90-117. Cambridge University Press, Cambridge, UK.

Westerbergh,A. & Saura,A. 1994. Gene flow and pollinator behaviour in *Silene dioica* populations. Oikos 71: 215-224.

Westerkamp,C. 1997. Flowers and bees are competitors- not partners.Towards a new understanding of complexity in specialised bee flowers. Acta Horticulturae 437: 71-74.

Westrich,P. 1990. Die Wildbienen Baden-Württembergs I+II. Eugen Ulmer Verlag, Stuttgart, Germany.

Westrich,P. 1996. Habitat requirements of central European bees and the problems of partial habitats. In: Matheson,A., Buchmann,S.L., O'Toole,C., Westrich,P. & Williams,I.H. (eds.), The conservation of bees, pp. 1-16. Academic Press, London, UK.

Wheeler,B.R. & Hutchings,M.J. 2002. *Phyteuma spicatum* L. Journal of Ecology 90: 581-591.

Widén,B. & Widén,M. 1990. Pollen limitation and distance-dependent fecundity in females of the clonal growth gynodioecious herb *Glechoma hederacea* (Lamiaceae). Oecologia 83: 191-196.

Wilcock,C. & Neiland,R. 2002. Pollination failure in plants: why it happens and when it matters. Trends in Plant Science 7: 270-277.

Williams,C.S. 1995. Conserving Europe's bees: why all the buzz? Trends in Ecology and Evolution 10: 309-310.

Williams,I.H. 1996. Aspects of bee diversity and crop pollination in the European Union. In: Matheson,A., Buchmann,S.L., O'Toole,C., Westrich,P. & Williams,I.H. (eds.), The conservation of bees, pp. 63-80. Academic Press, London, UK.

Williams,P.H. 1986. Environmental change and the distributions of British bumble bees (*Bombus* Latr.). Bee World 67: 50-61.

Willis,J.C. & Burkill,L.H. 1895. Flowers and insects in Great Britain. Part I. Annals of Botany 9: 227-267.

Wolf,A.T. & Harridson,S.P. 2001. Effects of habitat size and patch isolation on reproductive success of the Serpentine morning glory. Conservation Biology 15: 111-121.

Wynhoff,I., van Swaay,C.A.M., Brunsting,A.M.H. & van der Made,J.G. 2001. Conservation of *Maculinea* butterflies at landscape level. Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society (NEV) 12: 135-140.

Zar,J.H. 1984. Biostatistical analysis. Prentice-Hall, New Jersey, USA.

Summary

Introduction

Biodiversity in the sense of species richness may play a crucial role for maintaining ecosystem processes and therefore also for maintaining biodiversity itself. Pollination by animals is such a process. Worldwide species within nearly all taxonomical groups have declined or disappeared. This may be harmful for the functioning of ecosystems, thereby leading to further declines of biodiversity.

Biotic pollination involves two parties or communities, plants and animals, that mutually benefit from each other. Animals actively or passively transfer pollen grains needed for the pollination and seed set of plants. Plants provide animals with food, shelter or pheromones in the flowers. Pollination is important for the majority of wild plants and many crops: without pollination plant species and harvests will decline. This thesis is about the importance of biodiversity for the pollination of insect-pollinated plants.

Pollination systems can be classified into types of plant species with similar flowers that are visited by similar pollinator taxa. These types are called pollination syndromes. Plants that are pollinated by one or few pollinator species, and animals that confine their menu choice to one or few plant species, are called specialists. Plants pollinated by many pollinator species, and insects foraging on many plant species, are generalists. The majority of plant-pollinator interactions are unspecialised. The distribution of interactions between plants and flower visitors is asymmetrical: specialist plant species are visited by mainly generalist animals, and specialist animals visit mainly generalist plants. One-to-one relationships between single plant and animal species are extremely rare, particularly in temperate climates. In the study area of this thesis (the Netherlands, northwest Europe), only insects occur as pollinators. The majority belong to Diptera (flies, including syrphids), Lepidoptera (butterflies and moths) and Hymenoptera (mainly bees and bumblebees).

The role of plant and insect diversity for pollination is largely unknown. Plant diversity can have positive (facilitation) and negative (competition) effects on pollination of a plant species: e.g. several plant species together can attract more pollinators than one, but plants can also compete for pollinators. The higher the insect diversity, the higher will be the chance that a certain plant species is visited by its appropriate pollinator(s). Because most pollination interactions are unspecialised, the relation between diversity and pollination will not be straightforward: the extinction of a single species at one of the interacting levels does not directly lead to one extinction at the other level.

In Europe, including the Netherlands, most ecosystems are semi-natural and a result of centuries of extensive farming practices. During the second half of the 20th century, land use changed drastically and agriculture intensified. Many habitats declined, were deteriorated or became fragmented, resulting in the decline of plant and animal species. Two important flower-visiting insect taxa, butterflies and bees, are among the most negatively affected groups. In intensified agricultural landscapes, habitat remnants are important for preserving biodiversity outside natural reserves, and may be stepping stones between reserves. In the Netherlands, road verges, ditches and field margins are the main habitat remnants.

Questions and methods

The main question of this thesis was:

Is a high biodiversity important for the pollination of entomophilous wild plant species in agricultural, fragmented landscapes?

Biodiversity was studied in various ways, from landscape and community level to pollination and seed set at the level of individual species or patches. The complete sequence from insect visitation and behaviour, via actual pollination until seed set and germination was included. How finally seed set affects plant species numbers, and thus community composition, was out of the scope of this study.

The research area was in the north of the province of Drenthe in the Netherlands. Most of the research activities were performed in road verges and ditch banks, situated in matrices with various types of land use. Land use varied between heavily fertilised and sprayed intensive grassland and arable land, intensively or extensively grazed meadows, and semi-natural plant species-rich habitats (mostly in natural reserves of the stream valley of the Drentse Aa).

Both field surveys (descriptive analyses) and experiments (garden and field) were used. In the field surveys, the diversity of flowering plants and their flower visitors were measured with transects of road verges and ditch banks of 100 m. Insects on all flowers were counted every two weeks at 51 sites from May- October 2000 and at 18 sites from May-August 2001. The total number of flowering plant species found was 97 from 24 families in 2000 and 74 species from 20 families in 2001. The number of insect species was 361 from 9 orders in 2000, and 204 species from 8 orders in 2001. The large data set was used in the **chapters 2 - 5** and **8**. Experiments with potted plants of a number of selected species in 2000 - 2003 were used in the **chapters 5, 6** and **7**. With potted plants the number and arrangement of flowers can be controlled, and the plants can be placed into various environments. Furthermore, the growing circumstances are equal for the plants. Visitation rate (the number of visits a flower receives per unit time) and pollination, and seed set were measured in the experiments.

Landscape, plant and insect diversity, and flower visitation

In the first three chapters, processes were studied at community level. In chapter 2, the functional diversity of the plant and insect species was described: what types of pollination systems or syndromes can be found, and what are the degrees of specialisation of the plant species? The use and application of pollination syndromes is frequently criticised in literature: syndromes are often applied without field data, and syndromes are said to indicate that pollination interactions of plants and animals are specialised, while the majority are generalised. The goals were (1) to determine classes of plant species with similar frequencies of flower visiting insect taxa using cluster analysis, and (2) to investigate how existing pollination classifications are reflected in those classes. The majority of the plant species appeared to be generalists and not more than a third can be regarded as ecologically specialised. Fourteen classes could be identified. The proportion of flies, syrphids and solitary bees on the one hand, and the proportion of bumblebees, *Rhingia campestris* (a long-tongued syrphid) and honeybees on the other were the main characteristic insects for the two major two groups of classes. The accessibility of nectar and pollen was the most important feature that determined the differences of proportions of the visitor groups between the classes. Two of the known pollination syndromes were applicable for less than half of the plant species: the syndrome of flies (two classes with generalist plants) and the syndrome of bees (one class of specialist plants).

In **chapter 3** species richness and abundance of plants and insects were quantified in relation to the type and intensity of land use ("landscape diversity") and each other. The diversity of plants and solitary bees at a site was affected by the surrounding type of land use, while total insect diversity was not. Plant and solitary bee diversity were lowest at sites with the highest agricultural intensity. Insect diversity was positively related with plant diversity: the more plant species and flowers at a site, the more insect species and individuals could be found. Fragmentation and land use affect plant species richness and abundance, and together with site management these affect insect species richness and abundance. Particularly solitary bees are vulnerable.

Foodweb analysis can give insight in what may be the consequences of biodiversity loss for ecosystems. In **chapter 4** the effects of biodiversity on the frequency of interactions between flowering plants and flower visiting insects were described in such a foodweb context. The research question was: What is the effect of biodiversity on (1) connectance (he proportion of all possible interactions between plants and insects that are actually established), (2) the number of insect species that visit a plant species ("plant linkage level"), and (3) on the number of plant species an insect species visits ("insect linkage level")? The effects were analysed with path analysis.

The mean connectance per census was 0.21 ±0.01 SE, the mode 0.33. Plant species richness was the most important predictor in the path model. The mean plant linkage level per census was 2.27 ±0.09 SE and the mean linkage level was 35.0 ±3.9 SE. The mean insect linkage level per transect was 1.29 ±0.02 and the mean total linkage level was 15.9 ±1.3. Plant species richness was the most important predictor for connectance. There was a negative direct and positive indirect effect of plant diversity on the number of insect species that visit a plant species: on average, plants were visited by less insect species when there were more plant species. But there were more visitor species per plant species when insect species richness was higher. Insect species richness in turn was positively affected by plant species richness.

Insect linkage level varied much less, and the effect of plant species richness was positive: the more flower species are present in a vegetation, the more species an average insect species will visit. An important finding was that the abundance of a species (plants and insects) increased the chance for the number of interactions with other species.

Although this may sound rather trivial, it is often forgotten or doubted in literature. Furthermore, the effects varied in strength and direction between taxonomical and functional groups of plants and insects. In conclusion, human-induced differences in species richness of plants and insects do affect plant-flower-visitor networks. In literature so far the effect of biodiversity on connectance and linkage levels were only observed across natural biodiversity ranges at larger geographical and temporal scales.

Pollination and seed set of individual plant species

In the next three chapters the focus is on plant species rather than the entire community. The effects on pollination of plant diversity (**chapter 5**), insect diversity (c**hapter 6**), and of diversity in relation to plant population size (**chapter 7**) are studied.

In **chapter 5** the effects of a plant species' neighbouring plants on its visitation and pollination were analysed with six plant species from the large field data set, and a small garden experiment with Scabiosa columbaria. Contrary to chapter 4, the type and abundance of other flowering species in a community rather than plant species richness affected the number of visiting insect species per plant species (field data). Plants that are more similar interact more with each other. Analysing data of individual and only of generalist species may be the cause of this difference with chapter 4.

In the experiment with *S. columbaria* the effects of plant neighbourhood on visitation rate and pollination were analysed with patches of potted *Scabiosa* plants. In patches with high surrounding plant diversity, visitation was highest, but pollination quality lower than in a patch with a less diverse plant neighbourhood. Thus flower visitation rate alone may be misleading when one wants to evaluate facilitation or competition between plants: the resulting pollination and reproduction may be different and even opposite. The main conclusion from this chapter is that the balance between facilitation and competition between plants depends on plant population size and flower abundance of both the target and other plant species in the community, and on plant community species composition.

Chapter 6 is about the vulnerability to pollinator species loss of plant species with various degrees of specialisation. In other words, are pollinators equally exchangeable when biodiversity decreases for different plant species? And how important is insect pollination compared to self-pollination? A field experiment was conducted with generalist plants with generalist visitors (*Anthriscus sylvestris* and *Succisa pratensis*), specialist plants with generalist visitors (*Phyteuma spicatum* subsp. *nigrum* and *Scrophularia nodosa*), and specialist plants with specialist visitors (*Campanula rotundifolia* and *Lysimachia vulgaris*). Seed set of the plant species with potential self-pollination capacity was considerably reduced without insect visitation, indicating that self-pollination is a less secure solution for pollinator deficiency than is often assumed. For very common and extremely generalist plant species (here: Anthriscus), there will always be some visitors present acting as pollinators. The two specialist plant species with few specialist pollinators were the most vulnerable to pollinator loss: for *Campanula* and *Lysimachia* the absence of their oligolectic bees had a negative effect on visitation rate, seed set and germination. For the remaining generalist and specialist plant species with generalist pollinators, predictions are difficult to make.

In **chapter 7** the role of biodiversity for pollination is placed in a context of population characteristics and individual pollinator behaviour. The effects of plant and insect species richness and population size on the individual behaviour of insect visitors, insect visitation, pollen deposition and purity of deposited pollen were analysed for *Succisa pratensis*. Large syrphids (hoverflies) and bumblebees were by far the most important pollinators. Large populations of *S. pratensis* were visited by more insect species than small. Visitation rates did not differ between *S. pratensis* populations, but pollination quality and quantity were lowest in small pollinations. The size of *S. pratensis* populations was much more important than diversity of flowering plants and flower visiting insects. The individual behaviour of insects (flower constancy measured directly and indirectly by analysing pollen loads on insect bodies and the pollen deposited on stigmas of the flowers) was affected by population size, explaining the differences in pollination.

Andrena marginata, a specialist bee foraging on *S. pratensis*, is extinct in the Netherlands. If it was a better pollinator than syrphids and bumblebees, then the possible shift to a more generalist pollinator assemblage of syrphids and bumblebees had negative consequences for the pollination of *S. pratensis*. The abundance or absence of specialist pollinators can be good indicators of declining plant population sizes, because insects will react faster than plants.

Minimal insect diversity for the pollination of a plant community

During the field work for this thesis a few abundant insect species were repeatedly observed on many plant species. In **chapter 8** this was addressed quantitatively: Are most plant species in a community visited and pollinated by a small set of dominant insect species? The data set of the transect observations was used for analysing this. Indeed, only ten insect species (2.5% of the species observed!) were very often observed on most plant species: these insects constituted more than 50% of the visitors of two thirds of the analysed plant species. But if all plant species should get 50% and 75% of their visitors, respectively 39 and 93 of the observed insect species were needed. For a guaranteed pollination of the entire plant community the top-ten species are not enough. Firstly, some of the "top-ten" visitors are low quality pollinators. Secondly, more pollinator species are needed for the pollination of the plant community for reasons involving the degree of specialisation of plants and pollinators, variation in space and time, plant population size and density, and interactions between plants. For a sustainable minimal pollinator fauna, a good deal of habitat heterogeneity, continuous food supply throughout the flowering season and a diverse vegetation are needed. In summary, insect species that seem to be "important" as pollinators due to their frequencies (chapters 2-4), may appear to be less important or even negligible when the number of visits (the visitation rate) or other pollination qualities are considered.

Conclusions

The answer to the question about the importance of biodiversity for pollination differed between ecological levels: at plant community level, a high pollinator diversity can be important for the pollination of all plant species (chapters 2-4 and 8), while an individual plant species will often only need its particular pollinators (chapters 5-7). One important aspect, the individual behaviour of insects, got relatively little attention and was only directly measured in chapter 7. The behaviour of species does not necessarily give a clue about the individual behaviour. Scoring only frequencies can be misleading as an estimate of pollination quality, and visitation speed (the number of flowers an insects visits in a sequence) can provide more information. Similarly, the number of plant species an insect species visits (insect linkage level) do not tell how flower-constant an individual is. Frequencies are observations of single moments in time, while individual behaviour shows what happens in a continuous time period. The individual behaviour differs between species and even within species between individuals, partially depending on plant community composition.

BIODIVERSITY AND POLLINATION: PLANT SPECIES LEVEL

The importance of the diversity of flowering plants and flower-visiting insects for the reproduction and maintenance of a plant species depends on the context: the composition of the flower neighbourhood of the target species, the plant's pollination biology and life history (chapter 6), the degree of specialisation (chapters 4 & 6) and plant population characteristics, i.e. size, isolation and density (chapters 6 & 7). The type of hypothesis about the role of biodiversity for pollination of individual plant species or populations must be of the "context-dependence" type. Predicting the vulnerability of single plant species and populations to loss of biodiversity, particularly pollinators, can only be done by combining species- and site-specific data.

BIODIVERSITY AND POLLINATION: PLANT COMMUNITY LEVEL

The diversity of plants and insects is important for a guaranteed long-term pollination of an entire plant community. Many insect species are needed for the pollination, and these insects require a diverse plant community (chapter 8). However, this is not for all flower-visiting insects equally the case. For plant-pollinator communities it is more important that trophic interactions such as pollination take place, rather than which exact species is eating or pollinating which other species. This means that the functional diversity is more important than species diversity for species interactions and diversity relations. Thus for a plant community various insect groups, including generalist and specialists, need to be present. The continuous availability of a variety of various flower types is needed for maintaining a diverse pollinator assemblage. Also here a minimal level of functional (flower) types is required. Exceptional are strict specialists that will need their specific host or partner (e.g. monolectic bees, butterfly larvae and specialist plants). It seems likely that the role of biodiversity lies somewhere between all species and some keystone-species. A proposal for a name for such a hypothesis is "critical diversity level hypothesis".

POLLINATION, MANAGEMENT AND CONSERVATION

Mowing of road verges and ditch banks is a normal management measure. This has also been applied in the study sites of this thesis. Analysing the effect of the management (mowing) of road verges and ditch banks on pollination was not the goal of this thesis, but due to its frequent occurrence it could not be ignored. Data about the effects of mowing on flowering and flower visitation were shown in chapter 3 and **box 9.1**. The management and mowing regimes were extremely variable, and the frequency varied between 0- 6 times per site (box 9.1). Some parts of the research area where several verges were mown within the same period could be without any flowers for a week or more.

At sites in continuous agricultural areas often no alternative food sources in the time after mowing were available for the insects, as there were no flowers in the crop fields or intensively used grasslands. The effect of no alternative food sources was apparent for bees: the number of solitary bee species and individuals was significantly higher in unmown or mown sites with unmown patches or meadows in the vicinity, compared to mown sites without alternative foraging possibilities (chapter 3). The majority of the plant species could produce new flowers after mowing, but (much) less than before (box 9.1). The flower-visiting assemblage and the amount of visitation before and after mowing differed only for generalist plant species, but not for specialist plant species. In what way this may affect pollination and the reproductive output of a delayed or second flowering cannot be said from the data in this thesis.

In agricultural landscapes the direct effects from mowing, and indirect effects from land use, such as spraying and fertiliser input, should be tackled together. The type of management is crucial for the conservation of plants and animals. Which type of management is chosen depends on the conservation goals and other functions of a habitat. However, in some parts of northern Drenthe any management goals other than keeping the vegetation as short as possible seem to be lacking. Conservation in rural areas requires a better coordination and cooperation than currently is the case.

For the conservation of plants, particularly in fragmented habitats, the habitat requirements of pollinators and the phenology of both flowering plants and their pollinators are important criteria. Concerning mowing, plant and insect phenology are important criteria. If hay is not removed within two weeks after mowing or not at all, accumulation of nutrients can lead to plant species-poor vegetation types. A high plant diversity and flower availability are necessary flower visiting insects. For the plant communities studied, these insects are several groups of flies, syrphids, short- and long-tongued bumblebees, and polylectic and oligolectic solitary bees. Rare plant species may indirectly depend on common species, therefore also core and common plant species should be integrated in conservation. Increasing habitat heterogeneity in agricultural areas will have a positive effect on insect species richness. These insects are needed for pollination of wild plants, and several crops. A higher insect diversity also increases the chance for insects that can control pests and thereby contribute to biological pest control.

Samenvatting

Inleiding

Biodiversiteit in de zin van aantallen soorten kan een cruciale rol spelen voor processen in een ecosysteem. Aangezien wereldwijd de soortenrijkdom achteruitgaat, kan dat het functioneren van ecosystemen in gevaar brengen en zo tot verdere achteruitgang van soorten leiden. Bestuiving van planten is zo'n ecosysteemproces. Bij biotische bestuiving zijn twee partijen of gemeenschappen betrokken, planten en dieren. Deze kunnen wederzijds voordeel van elkaar hebben: dieren transporteren stuifmeelkorrels, noodzakelijk voor de bestuiving en zaadzetting van bloemplanten, en de planten voorzien deze dieren van o.m. voedsel en schuilplaatsen.

Dit proefschrift gaat over de rol van biodiversiteit voor de bestuiving van wilde planten als gemeenschappen en als afzonderlijke soorten, onderzocht in agrarische landschappen. In Noordwest-Europa zijn alleen insecten dierlijke bestuivers. De meeste soorten behoren tot de Diptera (vliegen inclusief zweefvliegen en muggen), Lepidoptera (dag- en nachtvlinders) en Hymenoptera (vliesvleugeligen, voornamelijk bijen en hommels). Gedurende de tweede helft van de twintigste eeuw werd in Europa de landbouw sterk geïntensiveerd. Als gevolg van de achteruitgang, versnippering en het verdwijnen van natuur zijn planten- en diersoorten achteruitgegaan. Twee bloembezoekende insectentaxa, vlinders en bijen, behoren tot de meest negatief beïnvloede groepen. Habitatresten(“natuursnippers”) in intensieve agrarische landschappen zijn belangrijk voor het behoud van biodiversiteit buiten beschermde natuurgebieden, en kunnen als verbinding tussen natuurgebieden functioneren. In Nederland zijn dat wegbermen, slootkanten en akkerranden.
De hoofdvraag van dit proefschrift is:
“Is een hoge biodiversiteit belangrijk voor de bestuiving van door insecten bezochte, wilde plantensoorten in agrarische en versnipperde landschappen?”

Over de rol van planten- en insectendiversiteit voor bestuiving is weinig bekend. Plantendiversiteit kan zowel een positief als een negatief effect hebben op bestuiving. Meer plantensoorten kunnen samen meer bestuivers aantrekken dan een enkele (facilitatie), maar ze kunnen elkaar ook beconcurreren om bestuivers (competitie). Hoe hoger de insectendiversiteit op een bepaalde plek is, des te groter wordt de kans dat daarbij ook de juiste bestuivers van een plant zijn. De meeste relaties tussen planten en hun bestuivers zijn niet gespecialiseerd, dus generalistisch; d.w.z. dat veel soorten insecten op een bepaalde plant terecht kunnen (vanuit de plant gezien) of dat een bepaalde insectensoort veel soorten planten bezoekt (vanuit het insect gezien).

Omdat de meeste bloembezoek- en bestuivingsinteracties ongespecialiseerd zijn, is de relatie tussen diversiteit en bestuiving niet direct. Het verdwijnen van één plantensoort leidt niet automatisch tot het verdwijnen van één insectensoort of omgekeerd. De verdeling van interacties tussen planten en bloembezoekers in een gemeenschap is over het algemeen asymmetrisch: specialistische planten worden eveneens door generalistische dieren bezocht, en specialistische dieren bezoeken ook vaak generalistische plantensoorten. Een op een relaties tussen een enkele plantensoort en een enkele diersoort zijn zeldzaam in gematigde klimaatzones, waartoe ook het onderzoeksgebied van dit proefschrift hoort. Om de bestuivingsrelatie tussen plantengemeenschappen en insectengemeenschappen te kunnen analyseren, was het noodzakelijk ook onderzoek te doen naar “bestuivingssyndromen”. Dit zijn combinaties van groepen plantensoorten met qua vorm en grootte vergelijkbare bloemen en de daarbij behorende groep bestuivers.

Methoden en aanpak

In het noorden van de provincie Drenthe zijn onderzoekslocaties uitgezocht die verschillen in soortenrijkdom van planten en insecten. Hiervoor zijn wegbermen en slootkanten gebruikt, die omgeven waren door verschillende typen landgebruik en daarmee samenhangende natuurlijkheid van het landschap. Het landgebruik varieerde van bemeste en met bestrijdingsmiddelen bespoten graslanden en akkers, intensief of extensief begraasde weilanden tot halfnatuurlijke en plantensoortenrijke gebieden (voornamelijk in het stroomdal van de Drentse Aa).

Om de processen die bij bestuiving een rol spelen te begrijpen, moet er vanuit het perspectief van zowel planten als insecten worden gekeken. Een methode waarbij gekeken wordt vanuit het insect is de *transectmethode*. In ongeveer 50 transecten (stroken in wegbermen en slootkanten) van 100 meter lengte werden van mei tot oktober in 2000 en 2001 om de twee weken alle bloemen en de insecten (bezoekers) op die bloemen geteld. Hiermee wordt in feite de bloemkeuze van insecten gemeten. Dit leverde een aanzienlijk gegevensbestand op: in het gebied werden 715.000 bloemen verspreid over 96 plantensoorten geteld en op die bloemen bijna 29.000 insecten, verspreid over meer dan 370 insectensoorten.

De *plotmethode* werd voor het perspectief van de plant gebruikt: gedurende een bepaalde tijd wordt voor een aantal bloemen (een plot of patch) het aantal insectenbezoeken, gebracht door de versschillende insectensoorten, geteld dat iedere bloem in die tijd ontvangt. Een maat daarvoor is de bezoekdruk, oftewel het aantal insectenbezoeken per bloem per tijdseenheid. Verder werden er veldexperimenten met planten in potten van een aantal plantensoorten gedaan. Met deze methode konden het aantal bloemen en de rangschikking ervan zelf worden bepaald en konden planten in verschillende omgevingen worden geplaatst. Bovendien zijn zo de groeiomstandigheden voor alle planten gelijk. In de verschillende experimenten werden bezoekdruk, bestuiving en zaadzetting gemeten.

Voor dit proefschrift zijn de bestuivingsprocessen eerst op het niveau van de plantengemeenschap, vervolgens op het niveau van individuele plantensoorten, en tenslotte weer op dat van de gemeenschap onderzocht. Eerst werd een beschrijving gemaakt van de soorten (mate van specialisatie en de syndromen) en werd de diversiteit van insecten en planten in relatie tot het landschap geanalyseerd. Daarvoor werden vooral gegevens van de transecten gebruikt. Daarna werden effecten van planten- en van insectendiversiteit op bezoek en bestuiving van individuele plantensoorten met behulp van experimenten onderzocht. Vervolgens is met één plantensoort als voorbeeld het belang van diversiteit afgezet tegen andere factoren. Tenslotte is weer voor de hele planten- en insectengemeenschap onderzocht wat de minimale bestuivers- en plantendiversiteit in het beschreven gebied zou kunnen zijn.

Landgebruik, planten- en insectendiversiteit en bloembezoek

In de eerste drie hoofdstukken werden processen op het niveau van de gemeenschap bestudeerd. In **hoofdstuk 2** is een beschrijving van de functionele diversiteit van planten en insecten gemaakt: welke bestuivingssyndromen komen er in het gebied voor, en in welke mate zijn de verschillende plantensoorten gespecialiseerd? Dergelijke gegevens kunnen maar ten dele uit de literatuur worden afgeleid omdat gegevens veelal ontbreken. Met een clusteranalyse van de transectgegevens zijn klassen van plantensoorten met gelijkende frequenties van bloembezoekende insectengroepen gemaakt en vergeleken met bestaande indelingen van bestuivingssystemen. De meerderheid van de plantensoorten bleek generalistisch te zijn en één derde was gespecialiseerd. Het aandeel van vliegen, zweefvliegen en solitaire bijen aan de ene kant, en van hommels, de langtongige Snuitzweefvlieg (*Rhingia campestris*) en de Honingbij (*Apis mellifera*) aan de andere kant was bepalend voor de twee hoofdgroepen van 14 gevonden klassen. De bereikbaarheid van nectar voor het insect was het belangrijkste kenmerk dat de verschillen tussen de klassen kan verklaren. Twee van de bekende bestuivingssyndromen waren van toepassing voor minder dan de helft van de plantensoorten: het “vliegensyndroom” (twee klassen met generalistische soorten) en het “bijensyndroom” (één klasse van specialistische soorten).

In **hoofdstuk 3** werden soortenrijkdom en de aantallen bloemen en insecten gekwantificeerd in relatie tot landgebruik. De totale insectendiversiteit was positief gerelateerd aan plantendiversiteit: hoe meer plantensoorten en bloemen er op een plek waren, des te meer insectensoorten en individuen werden er gevonden. Het landgebruik had geen invloed op deze relatie. Voor de bijen was dit anders: de soortenrijkdom van solitaire bijen evenals van bloeiende planten was gerelateerd aan het landgebruik in de omgeving. Diversiteit van planten en solitaire bijen waren het laagst wanneer de landbouwintensiteit het hoogst was. Conclusie: versnippering en landgebruik beïnvloeden plantensoortenrijkdom en bloemhoeveelheid. Samen met beheer beïnvloeden ze insectensoortenrijkdom en aantal.

In **hoofdstuk 4** werden de consequenties van biodiversiteitsverlies voor ecosystemen onderzocht door gebruik te maken van technieken die zijn ontwikkeld voor voedselweb-analyse. Daarvoor zijn de transectgegevens gebruikt. Twee belangrijke variabelen zijn onderzocht: voor planten het aantal insectensoorten waardoor een plantensoort gemiddeld wordt bezocht ("plant linkage level"), en voor insecten het aantal plantensoorten dat een insectensoort gemiddeld bezoekt (“insect linkage level"). De effecten van het aantal plantensoorten, het aantal bloemen, het aantal insectensoorten en het aantal individuen op deze variabelen werden geanalyseerd met behulp van "path analysis", een statistische methode waarmee effecten van verschillende met elkaar gecorreleerde factoren op een variabele geanalyseerd kunnen worden.

Er was een negatief direct en een positief indirect effect van plantendiversiteit op het aantal insectensoorten dat een plantensoort bezoekt: gemiddeld werden planten door minder insectensoorten bezocht wanneer er meer plantensoorten waren (direct). Tegelijkertijd waren er meer bezoekerssoorten per plantensoort als de insectensoortenrijkdom hoger was. Insectensoortenrijkdom werd weer positief beïnvloed door plantensoortenrijkdom (indirect).

Het gemiddelde aantal plantensoorten dat een insect bezoekt varieerde minder en het effect van het aantal plantensoorten was positief. Dus hoe meer plantensoorten er in een vegetatie zijn, des te meer plantensoorten zal een gemiddeld insect ook bezoeken. Deze reactie was het sterkst voor vliegen, zweefvliegen en hommels, het zwakst voor solitaire bijen. Een belangrijke bevinding was dat grote aantallen van een soort (bij zowel planten als insecten) de kans op interacties met andere soorten verhoogde. Dat wil zeggen, dat als een plantensoort meer bloemen heeft op een bepaalde plek, de kans groter wordt dat er meer insectensoorten op worden waargenomen. Voor insecten geldt het omgekeerde. Voor zowel planten als insecten was dit effect het sterkst voor generalistische soorten. Dit aantalseffect lijkt weliswaar erg triviaal, maar wordt vaak over het hoofd gezien in de literatuur.

De conclusie is dat antropogene (door mensen veroorzaakte) verschillen in soortenrijkdom van planten en insecten inderdaad plant-bloembezoeker-interacties (voedselwebben als het ware) beïnvloeden. Tot nu toe wordt in de literatuur alleen melding gemaakt van het effect van biodiversiteitverlies in meer natuurlijke ecosystemen of op grotere geografische schalen.

Bestuiving en zaadzetting van enkele plantensoorten

In de volgende drie hoofdstukken staan plantensoorten centraal in plaats van hele gemeenschappen. De effecten op bestuiving van plantendiversiteit (**hoofdstuk 5**), insectendiversiteit (**hoofdstuk 6**) en van diversiteit in relatie tot populatiegrootte van een plantensoort (**hoofdstuk 7**) komen aan bod.

In **hoofdstuk 5** werden de effecten van de buurplanten op bezoek van zes plantensoorten onderzocht. Uit de transectgegevens zijn soorten uitgezocht met voldoende bezoekgegevens op meerdere locaties in dezelfde bloeiperiode. Dit bleek alleen voor een aantal generalistische soorten het geval: drie schermbloemigen (Apiaceae) en drie gele composieten (Asteraceae). In tegenstelling tot de resultaten in hoofdstuk 4, werd het aantal insectensoorten per plantensoort hier beïnvloed door het type en de aantallen bloemen van andere plantensoorten in een gemeenschap en niet door de totale plantensoortenrijkdom. Vooral planten die meer op elkaar lijken hadden meer interacties met elkaar. Bijvoorbeeld Biggenkruid (*Hypochaeris radicata*) werd door minder insectensoorten bezocht naarmate er relatief meer bloemen van verwante soorten, zoals Havikskruiden (*Hieracium* spec.) waren. Dat gegevens van individuele en uitsluitend generalistische soorten zijn gebruikt kan de oorzaak zijn van het verschil in conclusie met hoofdstuk 4.

Verder is een experiment met Duifkruid (*Scabiosa columbaria*) gedaan. Daarbij zijn de effecten van de omgeving van een plant op bezoekdruk en bestuiving onderzocht met in het veld uitgezette potplanten. Bij een hoge plantendiversiteit in de omgeving werden bloemen het meest bezocht, maar was de kwaliteit van de bestuiving lager dan in een minder diverse omgeving. Onder kwaliteit wordt het aantal soorteigen stuifmeelkorrels dat een plant na een dag ontvangen heeft verstaan. De bezoekdruk alleen zou misleidend kunnen zijn, omdat facilitatie en competitie tussen plantensoorten een verschillende uitwerking kunnen hebben op de bestuiving en de voortplanting, misschien wel een effect met een tegengestelde richting dan bij bezoekdruk.

De belangrijkste conclusie in dit hoofdstuk is dat het evenwicht tussen facilitatie en competitie afhankelijk is van de populatiegrootte en de aantallen bloemen van zowel de doelsoort als de andere soorten in een vegetatie, en dus ook van de soortensamenstelling.

Hoofdstuk 6 gaat over de gevoeligheid van plantensoorten met verschillende mate van specialisatie voor het verlies van bestuivers. Met andere woorden: zijn voor verschillende plantensoorten bestuivers te vervangen door andere insectensoorten wanneer biodiversiteit af zou nemen? En hoe belangrijk is insectenbestuiving vergeleken met zelfbestuiving? Er is een veldexperiment gedaan met generalistische planten met generalistische bezoekers (Fluitenkruid, *Anthriscus sylvestris* en Blauwe knoop, *Succisa pratensis*), specialistische planten met generalistische bezoekers (Zwartblauwe rapunzel, *Phyteuma spicatum* subsp. *nigrum* en Knopig helmkruid *Scrophularia nodosa*), en specialistische planten met specialistische bezoekers (Grasklokje, *Campanula rotundifolia*, met enkele bijensoorten en Grote wederik, *Lysimachia vulgaris*, met slechts één bijensoort).

De zaadzetting van plantensoorten met (volgens de literatuur) mogelijkheid tot zelfbestuiving was veel lager zonder insectenbezoek dan die van planten met bezoek. Dit geeft aan dat zelfbestuiving een minder “veilige” oplossing voor een tekort aan bestuivers zou kunnen zijn dan wordt aangenomen. De resultaten lieten zien dat voor zeer algemene en extreem generalistische soorten (Fluitenkruid) er altijd wel een paar soorten bezoekers zullen zijn die als bestuiver kunnen functioneren. De twee specialistische soorten met zeer weinig specialistische bestuivers zijn het gevoeligst voor verlies van bestuivers: voor Grasklokje en Grote wederik had de afwezigheid van hun oligolectische (d.w.z. specialistische) bijen een sterk negatief effect op bezoekdruk, zaadzetting en zaadkieming. Voor de overige generalistische en specialistische plantensoorten waren voorspellingen moeilijk te doen doordat bestuiving van verschillende factoren afhangt, die lokaal sterk verschillen.

In **hoofdstuk 7** werd de rol van biodiversiteit voor bestuiving bekeken in een context van populatiekenmerken en individueel gedrag van insecten voor een generalistische plantensoort: Blauwe knoop (*Succisa pratensis*). De gevolgen van soortenrijkdom van planten en insecten en van populatiegrootte voor het individuele gedrag van bloembezoekers, op bloembezoek, stuifmeeldepositie en de zuiverheid van het afgezette stuifmeel werden onderzocht. Het individuele gedrag betrof hier bloemtrouw, gemeten door het volgen van insecten en door de stuifmeellading op het lichaam van insecten en het op de stempels afgezette stuifmeel te analyseren. Grote zweefvliegsoorten en hommels waren veruit de belangrijkste bezoekers. Grote populaties van Blauwe knoop werden door meer insectensoorten bezocht dan kleine. De bezoekdruk verschilde niet tussen de populatietypen, maar de bestuivingskwaliteit en -kwantiteit waren het laagst in kleine populaties. Met andere woorden: de *populatiegrootte* van Blauwe knoop was veel belangrijker dan de *diversiteit* van planten en insecten. De bloemtrouw (het individuele gedrag), werd ook vooral beïnvloed door populatiegrootte van Blauwe knoop. Dit kan de verschillen in bestuiving tussen populaties verklaren.

De Oranje zandbij (*Andrena marginata*), een specialistische bij die op Blauwe knoop foerageert, is in Nederland uitgestorven. Als ze (in het verleden) een betere bestuiver was dan zweefvliegen en hommels, dan heeft de verschuiving naar meer generalistische bestuivers nu als gevolg, dat veel stuifmeel van Blauwe knoop aan andere plantensoorten verloren gaat en dat er veel soortvreemd stuifmeel op de stempels van Blauwe knoop terecht komt. Dit kan de sterke achteruitgang van deze plant, veroorzaakt door verschillende factoren, hebben versterkt. Het aantal of de aanwezigheid van specialistische bestuivers zouden goede indicatoren voor een mogelijke versterkte achteruitgang van plantenpopulaties kunnen zijn, omdat deze insecten eerder reageren op veranderingen dan planten.

Minimale insectendiversiteit voor de bestuiving van een plantengemeenschap
Gedurende het veldwerk voor dit proefschrift is op veel plantensoorten een beperkt aantal zeer talrijke insectensoorten waargenomen. In hoofdstuk 8 is nader uitgezocht of de meeste plantensoorten in een gemeenschap bezocht worden door een kleine groep van dominante insectensoorten, en of deze soorten ook de belangrijkste bestuivers zouden kunnen zijn. Hun werkelijke betekenis voor de bestuiving is niet onderzocht. De transectgegevens uit hoofdstuk 2-4 werden gebruikt. Voor de analyse is een selectie gemaakt van plantensoorten met voldoende waarnemingen: 60 plantensoorten, bezocht door 397 insectensoorten. Slechts tien insectensoorten (2,5% van de 397) zijn erg vaak gezien. Tot deze “toptien” van echte generalisten behoren enkele vliegen, zweefvliegen, hommels en de honingbij. Deze soorten droegen bij aan meer dan 50% van het aantal bezoekers van 37 van de onderzochte 60 plantensoorten. Maar om alle plantensoorten 50% en 75% van hun bezoekers te laten hebben, zijn resp. 39 en 93 van de waargenomen insectensoorten nodig. Voor een gegarandeerde bestuiving van de plantengemeenschap als geheel zijn de “toptien-soorten” niet genoeg: ten eerste zijn sommige van die bezoekers slechte bestuivers (weinig soorteigen en/of veel soortvreemd stuifmeel wordt op de stempels afgezet) en ten tweede zijn er meer insectensoorten nodig, o.m. vanwege de mate van specialisatie van planten en insecten, variatie in ruimte en tijd, populatiegrootte- en dichtheid van planten en interacties tussen planten(soorten). Voor een minimale en duurzame fauna van insectenbestuivers zijn landschapsheterogeniteit, de aanwezigheid van bloemen als voedselvoorziening gedurende het hele seizoen en een diverse vegetatie nodig. Samengevat: sommige insecten die “belangrijk” lijken te zijn op grond van hun aantal individuen (hoofdstuk 2-4, transectgegevens) zouden minder belangrijk of geheel verwaarloosbaar kunnen zijn als het aantal bezoeken (de bezoekdruk per bloem, plot gegevens) en andere kwaliteiten van bestuivers (bijv. vliegafstanden, hoeveelheid afgezet stuifmeel) worden beschouwd.

Conclusies
Het antwoord op de vraag over het belang van biodiversiteit voor bestuiving verschilt per ecologisch niveau: op het niveau van de plantengemeenschap kan een hoge diversiteit van bestuivers belangrijk zijn voor de bestuiving van alle plantensoorten (hoofdstuk 2-4 en 8), terwijl een individuele plantensoort vaak genoeg zal hebben aan haar specifieke bestuivers (hoofdstuk 5-7). Eén belangrijk aspect, het individuele gedrag van insecten, kreeg relatief weinig aandacht en werd alleen direct gemeten in hoofdstuk 7. Het gedrag van een soort

geeft niet noodzakelijkerwijs ook een beeld van het gedrag van het individu. Het scoren van alleen soortfrequenties kan misleidend zijn als maat voor de kwaliteit en kwantiteit van bestuiving. Bezoeksnelheid (het aantal bloemen dat een insect achter elkaar per tijdseenheid bezoekt) kan meer informatie verschaffen. Ook het aantal plantensoorten dat een insect bezoekt ("insect linkage level") maakt niet duidelijk hoe trouw een individu is aan een bepaalde plantensoort. Frequenties en aantallen zijn momentopnamen, terwijl het individuele gedrag laat zien wat er in een langere tijdsperiode gebeurt. Het individuele gedrag verschilt tussen soorten maar ook tussen individuen binnen soorten, hetgeen deels afhangt van de samenstelling van een plantengemeenschap.

BIODIVERSITEIT EN BESTUIVING: HET NIVEAU VAN DE PLANTENSOORT

Het belang van de diversiteit van bloeiende planten en bloembezoekende insecten voor de voortplanting en handhaving van een plantensoort hangt af van de context: de soortsamenstelling van de buurplanten van de doelsoort, de bloembiologie en levensloop van de plant (hoofdstuk 6), de mate van specialisatie (hoofdstuk 4 en 6) en plantenpopulatiekenmerken, d.w.z. grootte, isolatie en dichtheid (hoofdstuk 6 en 7).

Het voorspellen van de kwetsbaarheid van individuele plantensoorten en populaties voor een verlies aan biodiversiteit, bijvoorbeeld van bestuivers, kan slechts worden gedaan door soort- en plaatsspecifieke gegevens te combineren.

BIODIVERSITEIT EN BESTUIVING: HET NIVEAU VAN DE PLANTENGEMEENSCHAP

De diversiteit van planten en insecten is belangrijk voor een gegarandeerde bestuiving op de lange termijn van een gehele plantengemeenschap. Vele insectensoorten zijn nodig voor de bestuiving, en deze insecten vereisen een diverse plantengemeenschap (hoofdstuk 8). Echter, dit is niet voor alle bloembezoekers in gelijke mate het geval. Voor gemeenschappen van planten en bestuivers is het meer van belang dat een trofische interactie, hier bestuiving, plaats vindt, dan precies welke soort welke andere soort bestuift. Dus voor interacties tussen soorten en diversiteitrelaties is de functionele diversiteit belangrijker dan soortdiversiteit. Met andere woorden, voor een plantengemeenschap moeten verschillende insectengroepen aanwezig zijn, specialisten en generalisten. Een langdurige beschikbaarheid van verschillende bloemtypen is noodzakelijk om een diverse gemeenschap van bestuivers te behouden. Ook voor insecten is een minimaal niveau van functionele diversiteit noodzakelijk, in dit geval dus van bloemtypen. Uitzonderlijk zijn strikte specialisten die hun specifieke partner nodig hebben (bijv. monolectische bijen, specialistische planten). Het is waarschijnlijk dat het belang van biodiversiteit voor bestuiving ligt tussen alle soorten en een paar "keystone- of sleutelsoorten" Een naam van zo'n hypothese zou "kritiek diversiteitsniveau-hypothese" kunnen zijn.

BESTUIVING, EN NATUURBEHEER EN -BEHOUD

Het maaien van wegbermen en slootkanten is een gangbare manier van beheer. Dit was ook het geval op de locaties van dit onderzoek. Het bestuderen van de effecten van de beheersmaatregelen was niet het doel van dit onderzoek, maar door het frequente optreden ervan kon het niet worden genegeerd. Gegevens over de effecten van maaien op de bloei en op bloembezoek kwamen aan bod in hoofdstuk 3 en box 9.1. Het beheer en het maairegime waren extreem variabel, de frequentie varieerde tussen 0 en 6 keer maaien per locatie per seizoen. Sommige delen van het gebied, waar de locaties in eenzelfde periode werden gemaaid, konden gedurende een week of langer geheel bloemloos zijn.

Op plekken in aaneengesloten landbouwgebied waren na een maaibeurt vaak geen alternatieve voedselbronnen voor insecten aanwezig, d.w.z. geen bloemen in de akkers of intensieve graslanden. Het gevolg van de afwezigheid van alternatief voedsel bleek duidelijk voor bijen: het aantal solitaire bijensoorten en individuen was significant hoger op niet-gemaaide plekken of gemaaide plekken met niet-gemaaide stukken of weilanden in de buurt, dan op gemaaide plekken zonder alternatieve foerageermogelijkheden (hoofdstuk 3). Van de 28 onderzochte plantensoorten produceerden 21 na maaien nieuwe bloemen, maar vaak (veel) minder dan ervoor (box 9.1).

Er was slechts een beperkte hoeveelheid gegevens om het effect van maaien op bloembezoek te kunnen analyseren. Dit is voor twee generalisten, Biggenkruid (*Hypocharis radicata*) en Zandblauwtje (*Jasione montana*), en twee specialisten, Smeerwortel (*Symphytum officinale*) en Rode klaver (*Trifolium pratense*) gedaan. De samenstelling van de bezoekende insecten en het aantal bezoekende individuen verschilde voor en na het maaien voor de twee generalistische soorten (andere samenstelling, op sommige plekken minder bezoekers) maar niet voor specialistische soorten. In hoeverre dit de bezoekdruk, bestuiving en voortplanting na herbloei beïnvloedt kan niet worden gezegd met de gegevens van dit proefschrift. Het kan negatief zijn: na maaien is er een lagere kans op bestuiving. Maar ook positief, want het bloeiseizoen voor herbloeiende planten wordt immers verlengd. Daarmee is er wellicht ook langer voedsel voor insecten beschikbaar. Verder onderzoek naar positieve en negatieve effecten van verschillende typen maaibeheer op bloembezoek, insecten en bestuiving is gewenst.

Het landgebruik en type beheer zijn cruciaal voor het behoud van planten en dieren, en biodiversiteit in het algemeen. De keuze van het type beheer hangt af van de doelen voor natuurbescherming en overige functies van een gebied. In sommige delen van Noord-Drenthe lijkt echter ieder doel anders dan het zo kort mogelijk houden van de bermvegetatie te ontbreken. Natuur- en landschapsbehoud in landelijke gebieden vergt een betere coördinatie en samenwerking van de verschillende partijen (gemeenten, agrariërs, natuurbeheerders etc.) dan nu het geval is.

Voor de bescherming van plantensoorten, zeker in versnipperde habitats, zijn de habitateisen van bestuivers en de fenologie van zowel bloemplanten als hun bestuivers belangrijke criteria voor natuurbeheer en -bescherming. Een hoge plantendiversiteit en beschikbaarheid van bloemen zijn nodig voor bloembezoekende insecten. Voor de plantengemeenschappen uit dit onderzoek zijn dat verscheidene groepen vliegen, zweefvliegen, kort- en langtongige hommelsoorten, en oligolectische en polylectische bijen. Omdat zeldzame plantensoorten indirect afhankelijk kunnen zijn van algemene plantensoorten, dienen dus ook algemene soorten te worden geïntegreerd in natuurbeschermingsmaatregelen. Het verhogen van habitatheterogeniteit (kleine landschapselementen, afgestemd beheer van wegbermen en slootkanten) in agrarische landschappen zal een positief effect hebben op insectensoortenrijkdom. Deze insecten zijn nodig voor de bestuiving van wilde plantensoorten.

Zusammenfassung

Einführung

Biodiversität als Artenzahl könnte von Entscheidender Bedeutung für Ökosystemprozesse sein. Die weltweit zurückgehende Artenvielfalt könnte das Funktionieren von Ökosystemen gefährden und damit zum weiteren Rückgang von Arten beitragen. Pflanzenbestäubung ist eine Ökosystemfunktion. Bei der biotischen Bestäubung sind zwei Parteien oder Gemeinschaften beteiligt, nämlich Pflanzen und Tiere, die meist wechselseitige Vorteile haben. Tiere transportieren Pollen, die für die Bestäubung und Befruchtung der Pflanzen notwendig sind, und Pflanzen, d. h. die Blüten, versehen Tiere mit u. a. Nahrung und Unterschlüpfen.

Diese Doktorarbeit handelt von der Rolle von Biodiversität bei der Bestäubung von Wildpflanzen als Gemeinschaft und als einzelne Arten, mit Agrarlandschaften als Untersuchungsgebiet. Im Nordwesten Europas sind Insekten die einzigen Bestäuber. Die meisten gehören zu den Dipteren (Fliegen einschließlich Schwebfliegen und Mücken), Lepidopteren (Tag- und Nachtfalter) und Hymenopteren (Hautflügler, vorwiegend Bienen und Hummeln). Während des 20. Jahrhunderts wurde die Landwirtschaft in Europa stark intensiviert. Der Rückgang, die Zerschneidung und das Verschwinden von natürlichen Räumen hatten einen Bestandsrückgang vieler Pflanzen- und Tierarten zur Folge. Zwei wichtige blütenbesuchende Insektentaxons, Tagfalter und Bienen, gehören mit zu den am stärksten beeinträchtigten Artgruppen. Habitatreste („Naturflecken“) in (intensiven) Agrarlandschaften sind wichtig für den Erhalt von Biodiversität außerhalb von Naturschutzgebieten und können eine Verbindung zwischen Naturschutzgebieten darstellen. In den Niederlanden gehören dazu vorwiegend Straßen-, Graben- und Ackerränder.

Die Hauptfrage dieser Doktorarbeit lautet:

„Ist eine hohe Biodiversität für die Bestäubung von entomophilen Wildpflanzenarten in zerschneideten Agrarlandschaften wichtig?“

Bis her ist wurde die Rolle, die Pflanzen- und Insektendiversität bei der Bestäubung spielen, kaum untersucht. Pflanzendiversität kann einen positiven sowie einen negativen Effekt auf Bestäubung vorzeigen. Mehrere Pflanzenarten gemeinsam können mehr Insektenarten herbeiziehen als eine einzelne Art (Fazilitation), aber sie können ebenfalls im Konkurrenzkampf um Bestäuber verwickelt sein. Je größer die Artenvielfalt von Insekten an einem bestimmten Ort ist, umso größer wird die Wahrscheinlichkeit, dass dabei die richtigen Bestäuber einer Pflanze sind. Die meisten Beziehungen zwischen Pflanzen und ihren Bestäubern sind nicht spezialistisch, daher also generalistisch. Damit wird gemeint, dass viele verschiedene Insektenarten eine bestimmte Pflanzenart besuchen können (aus Sicht einer Pflanze), oder dass eine Insektenart mehrere Pflanzenarten besucht (aus Sicht eines Insekts).

Weil die meisten Blütenbesuch- und Bestäubungswechselwirkungen nicht spezialisiert sind, ist die Beziehung zwischen Diversität und Bestäubung nicht direkt. Wenn eine Pflanzenart verschwindet, hat das nicht direkt das Verschwinden einer Tierart zur Folge, und umgekehrt. Die Verteilung von Wechselwirkungen zwischen Pflanzen und Blütenbesuchern ist vorwiegend asymmetrisch: Spezialistische Pflanzenarten werden ebenfalls von generalistischen Tieren besucht, während spezialistische Tiere auch oft generalistische Pflanzenarten besuchen. Eins-zu-eins-Beziehungen von einzelnen Pflanzenarten mit einzelnen Tierarten sind sehr selten, vor allem in gemäßigten Klimas, wozu auch das Forschungsgebiet dieser Dokterarbeit gehört. Damit Bestäubungsbeziehungen zwischen Pflanzengemeinschaften und Insektengemeinschaften analysiert werden konnten, war es erforderlich, "Bestäubungssyndrome“ zu untersuchen. Das sind Kombinationen von Gruppen von Pflanzenarten mit bezüglich Form und Größe vergleichbare Blüten sowie die dazugehörige Gruppe von Bestäuberarten.

Methoden und Vorgehensweise

Im Norden der niederländischen Provinz Drenthe wurden Untersuchungsorte ausgesucht, die sich in Pflanzen- und Insektenartenvielfalt unterscheiden. Diese befanden sich in Straßen- und Grabenrändern, die mit verschiedenen Landnutzungsarten umgeben waren. Die Landnutzung bestand aus gedüngten und mit Bekämpfungsmitteln bespritzen Äckern und Grünland, intensiv bis extensiv beweidete Wiesen sowie halbnatürliche, teilweise unter Naturschutz stehende Gebiete (vorwiegend im Stromgebiet der Drentse Aa).

Zum Verstehen der Bestäubungsvorgänge ist ein abwechselnder Blick aus Sicht der Pflanzen und aus Sicht der Tiere erforderlich. Eine Methode aus Sicht der Tiere ist die *Transektmethode*. In ca. 50 Transekten (Abschnitte von Straßen- und Grabenrändern) von 100 Metern Länge wurden von Mai bis Oktober 2000 und 2001 alle zwei Wochen alle Blüten und alle Insekten (Besucher) auf diesen Blüten gezählt. Dabei wurde eine umfangreiche Datei von Messwerten erhalten: im Gebiet wurden 715.000 Blüten, verteilt über 96 Pflanzenarten gezählt und auf diesen Blüten fast 29.000 Insekten, verteilt über mehr als 370 Insektenarten.

Die *Plotmethode* ist eine Methode aus Sicht der Pflanze: während einer bestimmten Zeit wird bei einer Gruppe von Blüten (ein Plot oder Beet) die Zahl der Besuche, die jede Blüte innerhalb dieser Zeit erhält, gezählt. Ein Maß dafür ist die Besuchsrate, d. h. die Zahl der Insektenbesuche je Blüte pro Zeiteinheit. Darüber hinaus wurden mit einigen Pflanzenarten Versuche mit eingetopften Pflanzen gemacht. Mit solchen Versuchen kann man selbst die Zahl und Anordnung von Blumen bestimmen sowie die Pflanzen in verschiedene Umgebungen stellen. Außerdem sind so für alle Pflanzen die Wachstumsverhältnisse gleich. In verschieden Versuchen wurden Besuchsrate, Bestäubung und Samensatz gemessen.

Für diese Dokterarbeit wurden die Vorgänge bei Blütenbesuch und Bestäubung erst auf dem Niveau der Pflanzengemeinschaft, anschließen auf dem Niveau einzelner Pflanzenarten und zuletzt wieder auf dem Gemeinschaftsniveau untersucht. Zuerst wurden die Arten beschrieben (Spezialisierungsgrad und die Syndrome) und wurde der Zusammenhang von Pflanzen- und Insektendiversität mit der Landnutzung untersucht. Dazu wurden vorwiegend die Transektdaten verwendet. Anschließend wurden mithilfe von Versuchen die Wirkungen von Pflanzen- und Insektendiversität auf den Blütenbesuch und die Bestäubung einzelner Pflanzenarten erforscht. Danach wurde die Bedeutung von Diversität im Verhältnis zu anderen Faktoren abgewogen, mit einer Pflanzenart als Beispiel. Schließlich wurde für die gesamte Pflanzen- und Insektengemeinschaft untersucht, was die Mindestartenvielfalt von Insekten und Pflanzen im Untersuchungsgebiet sein könnte.

Landnutzung, Pflanzen- und Insektendiversität und Blütenbesuch

In den ersten drei Kapiteln wurden Vorgänge auf dem Gemeinschaftsniveau untersucht. In **Kapitel 2** wurde eine funktionelle Beschreibung der Artenvielfalt von Pflanzen und Insekten erstellt: Welche verschiedenen Bestäubungssysteme oder –Syndrome befinden sich im Forschungsgebiet und was ist der Spezialisierungsgrad der verschieden Pflanzenarten? Derartige Informationen können nur beschränkt aus der Literatur erhalten werden, da dazu in vielen Studien Daten fehlen. Mithilfe von „Cluster Analysis" der Transektdaten wurden Klassen von Pflanzenarten mit ähnlich großen Häufigkeiten von blütenbesuchenden Insektengruppen gebildet. Diese Klassen wurden anschließend mit schon bekannten Bestäubungssystemen verglichen. Es stellte sich heraus, dass die meisten Pflanzenarten Generalisten und fast ein Drittel Spezialisten sind. Die Zahl der Fliegen, Schwebfliegen und Solitärbienen einerseits und der Hummeln, der langrüssligen Schnauzen-Schwebfliege (*Rhingia campestris*) sowie der Honigbiene (*Apis mellifera*) andererseits bestimmten vorwiegend die beiden Hauptzweige der 14 gefunden Klassen. Das wichtigste Merkmal als Erklärung für die Unterschiede zwischen den Klassen war die Erreichbarkeit von Nektar für Insekten. Zwei der bekannten Bestäubungssyndrome könnten angewandt werden: das „Syndrom der Fliegen" (zwei Klassen mit generalistischen Pflanzen) und das „Syndrom der Bienen" (eine Klasse mit spezialistischen Pflanzen).

In **Kapitel 3** wurden Artenvielfalt sowie Zahl von Blüten und Insekten im Zusammenhang mit Landnutzung quantifiziert. Die Gesamtinsektendiversität verhielt sich positiv zur Pflanzendiversität: je mehr Pflanzenarten und Blüten es an einer Stelle gab, umso mehr Insektenarten und Individuen wurden dort beobachtet. Die Landnutzung hatte keine direkte Wirkung auf diesen Zusammenhang. Für Wildbienen galt dies nicht: die Artenvielfalt von Solitärbienen sowie Blütenpflanzen hing mit der Landnutzung zusammen. Die Diversität dieser beiden Artgruppen war am niedrigsten wenn die landwirtschaftliche Intensität am stärksten war. Fazit: Landnutzung und Habitatzerschneidung beeinflussen Pflanzenartenvielfalt und Blütenzahl. Diese wirken wiederum gemeinsam mit der Bewirtschaftung der Böschungen auf die Artenvielfalt und Zahl der Insekten.

In **Kapitel 4** wurden Konsequenzen von Biodiversitätsverlust für Ökosysteme mit Methoden untersucht, die man zum analysieren von Nahrungsnetzen entwickelt hat. Dazu wurden die Transektdaten verwendet. Zwei variabeln waren wichtig: für Pflanzen die Zahl der Insektenarten, von denen eine Pflanzenart im Durchschnitt besucht wird („Plant Linkage Level") und für Insekten die Zahl der Pflanzenarten, die eine Insektenart im Durchschnitt besucht („Insect Linkage Level"). Effekte der Pflanzen- und Insektenartenvielfalt, der Blütenzahl, sowie der Zahl von Insektenindividuen auf die genannten variablen wurden mithilfe von „Path Analysis“ analysiert, einer statistischen Methode, die ermöglicht, Effekte von verschieden miteinander korrelierten Variabeln zu analysieren.

Festgestellt wurden ein negativer, direkter sowie ein positiver, indirekter Effekt der Pflanzendiversität auf die Zahl der Insektenarten, von denen eine Pflanzenart besucht wird. Das heißt, durchschnittlich wurden Pflanzen von weniger Insektenarten besucht, je mehr Pflanzenarten es an einer Stelle gab (direkt). Zugleich gab es aber mehr Insektenarten je Pflanzenart wenn die Insektenartenvielfalt größer war. Die Insektenvielfalt wird wiederum positiv durch die Pflanzenartenvielfalt beeinflusst (indirekt).

Die durchschnittliche Zahl von Pflanzenarten, die eine Insektenart besucht, variierte weniger und der Effekt der Pflanzenvielfalt war positiv. Also je mehr Pflanzenarten sich in einer Vegetation befinden, umso mehr Pflanzenarten wird ein Insekt im Durchschnitt besuchen. Diese Reaktion zeigte sich am stärksten bei Fliegen, Schwebfliegen und Hummeln und am schwächsten bei Solitärbienen. Ein wichtiger Befund war, dass die Blüten- oder Individuenzahlen einer Art (also bei Pflanzen und Insekten) die Wahrscheinlichkeit von Interaktionen mit anderen Arten vergrößerte. Das heißt, dass eine höhere Blütenzahl einer Pflanzenart an einer bestimmten Stelle die Wahrscheinlichkeit erhöht, dass mehr Insekten beobachtet werden. Für Insekten gilt dies ebenfalls umgekehrt. Dieser Effekt mag zwar sehr trivial erscheinen, wird aber in der Literatur oft übersehen. Bei Pflanzen sowie Insekten war der Effekt bei generalistischen Arten am stärksten.

Aus diesem Kapitel lässt sich schließen, dass anthropogene (also von Menschen verursachte) Unterschiede der Artenvielfalt von Pflanzen und Insekten tatsächlich Wechselbeziehungen von Pflanzen und Blütenbesucher (also quasi Nahrungsnetze) beeinflussen. Bis her wurde in der Literatur nur von Biodiversitätseffekten in natürlichen Ökosystemen oder über größere geografische Maßstäbe berichtet.

Bestäubung und Samensatz einzelner Pflanzenarten

In den nächsten drei Kapiteln stehen statt der ganzen Artengemeinschaft, einzelne Pflanzenarten im Mittelpunkt: die Folgen der Pflanzendiversität (K**Kapitel 5**), der Insektendiversität (**Kapitel 6**) und des Zusammenhangs zwischen Diversität und Populationsumfang einer Pflanzenart (**Kapitel 7**) für die Bestäubung.

In **Kapitel 5** wurden Effekte von Pflanzen in der „Nachbarschaft“ auf Blütenbesuch von sechs Pflanzenarten untersucht. Aus den Transektdaten wurden Arten mit ausreichend Besucherdaten von verschiedenen Orten und innerhalb einer Blüteperiode ausgewählt. Dies war bei sechs generalistischen Arten der Fall: drei Doldenblütler (Apiaciaceae) und drei Korbblüter (Asteraceae). Im Gegensatz zu den Ergebnissen in Kapitel 4, wurde die Zahl der besuchenden Insektenarten pro Pflanzenart hier von den Typen und der Blütenzahl anderer Pflanzenarten in einer Gemeinschaft statt der gesamten Artenvielfalt beeinflusst. Wechselwirkungen waren vor allem zwischen ähnlichen Pflanzenarten häufiger. Zum Beispiel, je größer die relative Blütenzahl von verwandten Arten wie Habichtskräutern (*Hieracium* spec.) war, desto weniger Insektenarten besuchten Gewöhnliches Ferkelkraut (*Hypochaeris radicata*). Die Anwendung von Daten von einzelnen und ausschließlich generalistischen Arten könnte der Grund für den Unterschied zu den Ergebnissen in Kapitel 4 sein.

Darüber hinaus wurde in Kapitel 5 ein kleiner Gartenversuch mit Tauben-Skabiose (*Scabiosa columbaria*) durchgeführt. Dabei wurden Effekte der Umgebung einer Pflanze auf die Besuchsrate und Bestäubung mit eingetopften Pflanzen im Feld untersucht. In einer Umgebung mit großer Pflanzenartenvielfalt bekamen die Blüten die meisten Besuche, dagegen war die Bestäubungsqualität geringer im Vergleich zu Blüten in einer weniger artreichen Umgebung. Mit Qualität wird hier die Zahl der Arteigenen Pollenkörner gemeint, die eine Pflanze innerhalb eines Tages erhält. Die Besuchsrate ohne andere Daten könnte irreführen, da Fazilitation und Konkurrenz zwischen Pflanzenarten die Bestäubung und Fortpflanzung unterschiedlich bewirken könnten, eventuell sogar in eine der Besuchsrate entgegengesetzte Richtung.

Die wichtigste Schlussfolgerung dieses Kapitels ist, dass das Gleichgewicht zwischen Fazilitation und Konkurrenz vom Populationsumfang und der Blütenmenge von sowohl der Zielart wie den anderen Arten in der Vegetation sowie auch der Artenzusammensetzung bestimmt wird.

Kapitel 6 handelt von der Empfindlichkeit für den Verlust von Bestäubern bei Pflanzenarten mit verschiedenen Spezialisierungsgraden. Mit anderen Worten: Können die Bestäuber verschiedener Pflanzenarten bei Biodiversitätsabnahme durch andere Insektenarten ersetzt werden? Und wie wichtig ist Insektenbestäubung im Vergleich zur Selbstbestäubung? Ein Feldversuch wurde durchgeführt mit generalistischen Pflanzen mit generalistischen Besuchern (Wiesen-Kerbel, *Anthriscus sylvestris* und Gewöhnlicher Teufelsabbiss, *Succisa pratensis*), spezialistischen Pflanzen mit generalistischen Besuchern (Schwarze Teufelskralle, *Phyteuma spicatum* subsp. *nigrum* und Knotige Braunwurz, *Scrophularia nodosa*), sowie spezialistische Arten mit spezialistischen Besuchern (Rundblättrige Glockenblume, C*ampanula rotundifolia*, mit einigen Bienenarten und Gemeiner Gilbweiderich, *Lysimachia vulgaris*, mit nur einer einzigen Bienenart)

Der Samensatz von Pflanzenarten mit (der Literatur nach) Möglichkeit zur Selbstbestäubung war ohne Insektenbesuch um vieles geringer im Vergleich zu den Pflanzen, bei denen der Insektenbesuch nicht verhindert wurde. Das bedeutet, dass Selbstbestäubung eine weniger „sichere“ Lösung bei Bestäuberdefiziten sein könnte, als man glaubt. Die Ergebnisse zeigten, dass es für allgemeine und extrem generalistische Arten (Wiesen-Kerbel) immer irgendwelche Besucherarten geben wird, die als Bestäuber auftreten können. Die beiden spezialistischen Arten mit spezialistischen Bestäubern waren am empfindlichsten: bei der Rundblättrigen Glockenblume und dem Gewöhnlichen Gilbweiderich bewirkte die Abwesendheit ihrer oligolektischen (also spezialistischen) Bienen eine starke Verringerung der Besuchsrate, des Samensatzes und der Samenkeimung. Für die übrigen generalistischen und spezialistischen Arten sind Vorhersagen schwierig, weil Bestäubung von örtlich (stark) verschiedenen Faktoren bewirkt wird.

In **Kapitel 7** wurde die Rolle von Biodiversität bei der Bestäubung im Zusammenhang mit Populationsmerkmalen und individuellem Insektenverhalten anhand einer generalistischen Pflanzenart, Gewöhnlicher Teufelsabbiss (*Succisa pratensis*), betrachtet. Die Effekte von Pflanzen- und Insektenartenvielfalt sowie von Populationsgröße auf das Verhalten von einzelnen Blütenbesuchern, Blütenbesuch sowie die Pollenabsetzung und dessen Reinheit wurden untersucht. Das Einzelverhalten beinhaltet hier Blütentreue, die anhand von Insektenbeobachtungen sowie von Analysen der Pollenladungen der Insektenkörper und der Blütennarben ermittelt wurde. Große Schwebfliegenarten und Hummeln waren die wichtigsten Besucher. Große Teufelsabbisspopulationen wurden von mehr Insektenarten besucht als kleine. Die Besuchsraten waren zwischen den Populationstypen nicht verschieden, die Bestäubungsqualität- und -quantität hingegen waren am geringsten in kleinen Populationen. Das bedeutet also, dass die *Populationsgröße* des Teufelsabbiss viel wichtiger war als die *Diversität* der Insekten und Pflanzen. Blumentreue (das Einzelerhalten) wurde ebenfalls vorwiegend von der Populationsgröße beeinflusst, was die Bestäubungsunterschiede zwischen den Teufelsabbisspopulationen erklären könnte.

Die Hellrote Sandbiene (*Andrena marginata*), eine oligolektische (spezialistische) Bienenart, ist in den Niederlanden ausgestorben. Wenn sie (in der Vergangenheit) eine bessere Bestäuberart war als Schwebfliegen und Hummeln, hatte die Verschiebung in die Richtung der jetzigen generalistischeren Arten zur Folge, dass viele Pollen des Teufelsabbiss an andere Pflanzenarten verloren gehen und das viele artfremde Pollen auf die Narben des Teufelsabbiss landen. Dies könnten den starken Rückgang dieser Pflanzenart, der verschiedene Gründe hat, verstärkt haben. Die Zahl oder Anwesendheit von spezialistischen Bestäuberarten könnten gute Hinweise für einen möglichen verstärkten Rückgang von Pflanzenpopulationen sein, da diese Insekten eher auf Veränderungen reagieren als Pflanzen.

Mindestdiversität von Insekten für die Bestäubung einer Pflanzengemeinschaft

Während der Feldarbeit dieser Untersuchungen sind auf vielen Pflanzenarten einige wenige, aber sehr zahlreiche Insektenarten beobachtet worden. In **Kapitel 8** wurde eingehender untersucht, inwieweit die meisten Pflanzenarten innerhalb einer Gemeinschaft von einer kleinen Gruppe dominanter Insektenarten besucht wurden und ob diese Arten auch die wichtigsten Bestäuber sein könnten. Ihre tatsächliche Bedeutung bei der Bestäubung wurde nicht untersucht. Die Transektdaten der Kapitel 2-4 wurden dazu verwendet. Für die Auswertung wurden Pflanzenarten mit genügend Daten ausgewählt: 60 Pflanzenarten, die von 397 Insektenarten besucht wurden. Nur zehn Insektenarten (2,5 % von 397) wurden sehr häufig beobachtet. Zu dieser „Top Zehn" richtiger Generalisten gehören einige Fliegen, Schwebfliegen, Hummeln und die Honigbiene. Bei 37 aus 60 untersuchten Pflanzenarten trugen diese Arten zu mehr als 50 % der Zahl der Besucher bei. Aber wenn alle Pflanzenarten 50 % und 75 % ihrer Besucher haben sollen, werden 39 bzw. 93 der beobachteten Insektenarten benötigt. Für die Gewährleistung der Bestäubung einer gesamten Pflanzengemeinschaft reichen die „Top-Zehn-Arten" nicht aus. Erstens sind einige dieser Arten ungünstige Bestäuber (wenig arteigene Pollen und/oder viel artfremde Pollen werden auf die Narben gebracht) und zweitens werden mehr Arten benötigt wegen u. a. des Spezialisierungsgrads der Pflanzen und Insekten und Wechselwirkungen zwischen Pflanzen(arten). Für die Mindestinsektenfauna für eine nachhaltige Bestäubung sind Landschaftsheterogenität, genügend Blüten als Nahrungsquelle das ganze Jahr hindurch und eine vielfältige Vegetation erforderlich. Fazit: Manche Insektenarten, die aufgrund ihrer Indviduenzahl als „wichtig" erscheinen (Kapitel 2-4, Transektdaten) könnten weniger wichtig oder gar unbedeutend sein, wenn die Zahl der Besuche (Besuchsrate pro Blüte, Plotdaten) und andere Bestäuberqualitäten (z. B. Flugabstände, Menge der aufgebrachten Pollen) betrachtet werden.

Schlussfolgerungen

Die Antwort auf die Frage bezüglich der Bedeutung von Biodiversität für Bestäubung von Wildpflanzen ist je nach ökologischem Niveau verschieden. Auf dem Niveau einer Pflanzengemeinschaft kann eine hohe Insektendiversität für die Bestäubung aller Pflanzenarten wichtig sein (Kapitel 2-4 und 8), während bei einer einzelnen Pflanzenart die spezifischen Bestäuberarten genügen (Kapitel 5-7). Ein wichtiger Aspekt, das Einzelverhalten von Insekten, erhielt relativ wenig Aufmerksamkeit. Das Verhalten einer Art verrät nicht unbedingt wie sich ein einzelnes Insekt verhält. Nur Arthäufigkeiten messen könnte irreführend wirken als Maß für Bestäubungsqualität und -Quantität. Besuchsgeschwindigkeit (die Zahl der Blüten, die ein Insekt innerhalb einer bestimmten Zeit nach einander besucht) enthält mehr Informationen. Die Zahl der Pflanzenarten die eine Insektenart besucht (Insect Linkage Level) zeigt ebenfalls nicht die Blütentreue eines einzelnen Insekts. Häufigkeiten sind Momentaufnahmen, während das Einzelverhalten zeigen kann, was innerhalb eines längeren Zeitablaufs geschieht. Das Einzelverhalten ist je nach Insektenart sowie auch zwischen Individuen innerhalb einer Art verschieden und hängt zum Teil von der Zusammensetzung einer Pflanzengemeinschaft ab.

Biodiversität und Bestäubung: das Niveau der Pflanzenart

Die Bedeutung der Vielfalt von Blütenpflanzen und blütenbesuchenden Insekten für die Fortpflanzung und den Erhalt einer Pflanze wird vom Kontext bestimmt: die Artenzusammensetzung der Nachbarspflanzen der Zielart, die Blütenbiologie und der Lebensablauf der Pflanze (Kapitel 6), der Spezialisierungsgrad (Kapitel 4 und 6) und Populationsmerkmale, d. h. Größe, Isolierung und Dichte (Kapitel 6 und 7).

Vorhersagen über die Empfindlichkeit einzelner Arten und Populationen für Biodiversitätsverlust sind nur möglich, wenn Art- und Ortspezifische Daten gemeinsam betrachtet werden.

Biodiversität und Bestäubung: das Niveau der Pflanzengemeinschaft

Für eine nachhaltige Bestäubung einer ganzen Pflanzengemeinschaft ist die Diversität von Pflanzen und Insekten von großer Bedeutung. Für die Bestäubung sind viele Insekten erforderlich und diese Insekten benötigen eine vielfältige Pflanzengemeinschaft (Kapitel 8). Dies ist allerdings nicht für alle Blütenbesucher gleichermaßen der Fall. Für Pflanzen- und Bestäubergemeinschaften ist eher wichtig, dass die trophische Wechselbeziehung, also Bestäubung, statt findet als welche Art genau welche bestäubt. Also für Wechselbeziehungen zwischen Arten und bei Diversitätsbeziehungen ist die funktionelle Diversität wichtiger als die Artendiversität. Das bedeutet, dass für eine Pflanzengemeinschaft verschiedene Insektengruppen, Spezialisten und Generalisten, zur Verfügung stehen müssen. Für den Erhalt einer vielfältigen Bestäubergemeinschaft ist die dauernde Verfügbarkeit verschiedener Blütentypen eine Voraussetzung. Für Insekten besteht daher ebenfalls ein Mindestniveau funktioneller Diversität (von Blütentypen). Eine Ausnahme sind Spezialisten, die nur ihren besonderen Partner benötigen (z. B. monolektische Bienen, spezialistische Pflanzen). Allgemein kann davon ausgegangen werden, dass die Bedeutung von Biodiversität für Bestäubung zwischen allen Arten und einigen „Keystone- oder Schlüsselarten" liegt. Eine dazugehörige Hypothese könnte „Kritisches Diversitatsniveau-Hypothese" lauten.

Bestäubung: Naturerhalt und Naturbewirtschaftung

Eine übliche Bewirtschaftungsweise von Straßen- und Grabenrändern ist Mähen, was ebenfalls für das Forschungsgebiet galt. Die Folgen von Bewirtschaftungsweisen zu untersuchen war zwar nicht Ziel dieser Arbeit, sie konnten aber wegen der regelmäßigen Mahd nicht ignoriert werden. In Kapitel 3 sowie in **Box 9.1** wurden Ergebnisse bezüglich der Bewirtschaftung gezeigt. Die Bewirtschaftungsweise war sehr variabel, die Mähfrequenz schwankte zwischen 0 und 6 mal mähen pro Jahr pro Stelle. Einige Teile des Forschungsgebiets, wo viele Stellen zur selben Zeit gemäht wurden, waren nach der Mahd während einer Woche oder länger völlig Blütenleer.

An Stellen in ununterbrochenen Landwirtschaftsflächen gab es oftmals keine alternativen Nahrungsquellen für Insekten, d. h. keine Blumen in den Äckern oder im Grünland. Die Wirkung des Mangels an alternativen Nahrungsquellen war bei Bienen bemerkbar: die Zahl der Solitärbienenarten und Individuen war im Vergleich zu gemähten Stellen ohne alternative Nahrungsquellen bedeutend höher als an nicht-gemähten Stellen oder gemähten Stellen mit nicht-gemähten Wiesen in der Nähe (Kapitel 3). Nach der Mahd konnten 21 aus 28 untersuchten Pflanzenarten neue Blüten bilden, allerdings oft (viel) weniger als vorher (Box 9.1).

Zum Auswerten des Mäheffekts auf Blütenbesuch stand nur eine beschränkte Zahl von Messwerten zur Verfügung. Werte zweier Generalisten, Gewöhnliches Ferkelkraut (*Hypochaeris radicata*) und Berg-Sandknöpfchen (*Jasione montana*) sowie zweier Spezialisten, Gewöhnlicher Beinwell (*Symphytum officinale*) und Rotklee (*Trifolium pratense*) wurden analysiert. Bei den beiden generalistischen Arten waren die Zusammensetzung der besuchenden Insektenarten und die Individuenzahl vor und nach dem Mähen verschieden (an einigen Stellen weniger Insekten nach der Mahd). Bei den Spezialisten gab es keine Unterschiede. Inwieweit Besuchsrate, Bestäubung und Fortpflanzung durch die Mahd beeinflusst werden, kann aus den vorhandenen Messwerten nicht geschlossen werden. Eingehendere Untersuchungen in diesem Bereich sind erwünscht.

Landnutzung und Bewirtschaftungsweise sind entscheidend beim Erhalt von Pflanzen und Tieren sowie von Biodiversität generell. Entscheidungen über die Bewirtschaftungsweise werden von den Naturschutzzielen und von anderen Funktionen eines Gebiets bestimmt. In einigen Teilen von Norddrenthe scheint es allerdings so, dass es anderen Zielen als die Vegetation in Straßenrändern so kurz wie möglich zu halten, mangelt. Für den Natur- und Landschaftserhalt in ländlichen Gegenden sind eine bessere Koordination und Zusammenarbeit der Verschiedenen Teilhaber (Kommunen, Landwirte, Naturschützer usw.) erforderlich als gegenwärtig der Fall ist.

Zum Schutz von Pflanzenarten, besonders in zerschneideten Biotopen, sind die Habitatanforderungen der Bestäuber und die Phänologie der Pflanzen sowie der Insekten wichtige Naturbewirtschaftungskriterien. Eine hohe Artenvielfalt von Pflanzen und die Verfügbarkeit von Blüten sind notwendig für Insekten. Für die Pflanzen dieser Dokterarbeit sind verschiedene Fliegengruppen, Schwebfliegen, kurz- und langrüsslige Hummeln sowie oligo- und polylektische Bienen wichtige Insekten. Da seltene Pflanzenarten indirekt auf allgemeine Pflanzenarten angewiesen sein können, sollten auch allgemeine Arten in Naturschutzmaßnamen integriert werden. In Agrarlandschaften wird eine erhöhte Habitatheterogenität (kleine Landschaftselemente, zielgerechte Bewirtschaftung von Straßen- und Grabenrändern) eine positive Wirkung auf die Insektenvielfalt haben. Diese Insekten sind für eine erfolgreiche Bestäubung von Wildpflanzen unentbehrlich.

Epilogue and Acknowledgements

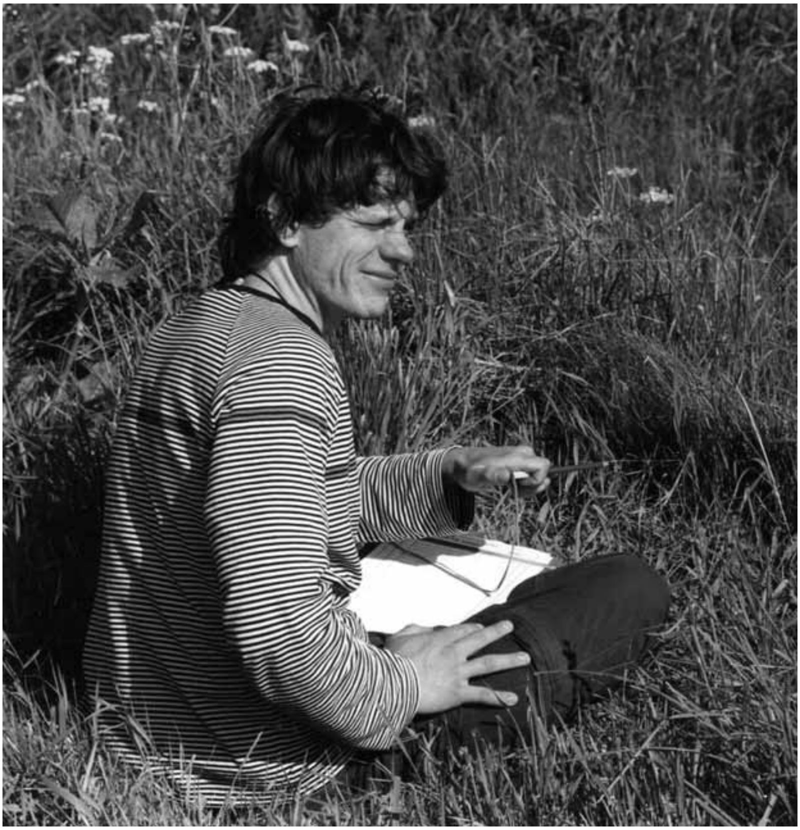
Biological research involves a lot of counting. For the present thesis this meant counting many flowers, insects, species, visits, and pollen grains. The subject of this thesis, insect pollination, is a sunny one, ideal for good weather biologists (many insects don't fly in bad weather). A disadvantage, however, was the northwest European climate, which is not always sunny, but frequently cloudy or rainy. Weather changes were sometimes frustrating – the fieldwork thus involved a fair amount of flexibility. Nevertheless it was fun to do. The biological variation I observed during the past years was fantastic! Variation was a central theme in this thesis, as it dealt with a hot topic in ecology: biodiversity. This term is used a lot, but also misused. It can be a magic word for grants and is a handy tool for many other purposes, as everything related to living beings or matter may be shovelled under the name biodiversity. However, for this thesis biodiversity was used in a pure meaning, i.e. species richness and abundance. Biodiversity is thought to be important for ecosystem functioning. But it is only recently that the number of studies with evidence in favour of or against this idea has started to increase. During the past years I also tried to add some information in this field: the role of biodiversity for pollination of wild plant species.

For me the project began at the start of the field season in 2000: I went almost straight into the field. The fieldwork during the first year was mainly meant to get an idea of the study area and to measure how species was distributed. The data were meant for one chapter. But the extensive data set (a result of weeks and weeks of counting insects and flowers...) provided opportunities for more papers. Apart from landscape and land use effects on diversity (chapter three) it was interesting and useful to characterise the pollination systems in the study area: chapter two was born. Some papers in literature had come up with the idea of a "foodweb approach" towards community pollination. After some doubts, the "Mother Nature" symposium in Århus provided the spark to actually launch chapter four. Well, a stay in a picturesque environment high up in the French Vosges Mountains also helped to get a clear vision. Chapter five narrows down to plant species level, and chapter six is the large experimental chapter. The plants for these experiments were stubborn and all wanted their individual treatment: in the first year (2002) several species did not flower or did this at a different time than their natural phenology. In 2003 they were mostly behaving properly again. But then some species had not survived winter, narrowing down the choice to the six species finally used. This is why so many plants and species were grown (and maybe this provides an explanation for those who wondered why I used so much space in the experimental garden). Experience at the institute with *Succisa pratensis* and the closely related *Scabiosa columbaria*, together with their useful flower biology, made them ideal model species for chapters five and seven. In chapter eight I used the large data set again and arranged it as the last data chapter to go back from individual species to the community. Unintended, this thesis has expanded "by itself". All in all the past five years were interesting, painstaking, sometimes shocking (to see what still is done to landscape and nature), frustrating, funny but mostly very enjoyable! However, the counts, analyses, discussions, advise, identifications, and several other activities were not possible without the helping hands, minds and mouths of many of you. So I would very much like to say

thanks bedankt danke dziękuję tack tak takk kiitos merci todah

to:
Manja Kwak, veel dank voor je rol als begeleider en coach. Je hebt steeds een belangrijke, ondersteunende en vooral enthousiasmerende rol gespeeld. Ook als het tegenzat benadrukte je de positieve dingen, en ondanks dat je geregeld ziek was, ben je toch vaak mee het veld in geweest en heb je stukken gelezen. Dit getuigt van een sterk karakter en onverwoestbaar doorzettingsvermogen. Je behoorlijk kritische houding en oog voor detail waren niet altijd makkelijk, maar wel nuttig en hielden het niveau omhoog. Het waren leuke jaren met prima begeleiding en prettige samenwerking! Jelte van Andel, als promotor heb je steeds de grote lijn bewaakt. Je interesse en belangstelling voor het onderwerp heb ik zeer gewaardeerd. Discussies met jou hebben tot vele nieuwe inzichten geleid. Je enthousiasme lijkt haast onuitputtelijk! Jacob Hogendorf, zonder je assistentie met materieel en kennis, en de verzorging van de planten in de proeftuin en de kas zou een groot deel van het werk onmogelijk zijn geweest. Je wist overal nog wel wat vandaan te toveren, en eigenlijk was niets te gek om uit te voeren. Bedaankt veur aal joen hulp! Maaike de Vlas, Iwona Kołodziejska, Carola van der Muren, Henk Hunneman, and Maaike Smelter: I would like to thank you as MSc students for your fieldwork, refreshing ideas and enthusiasm – Ik wil jullie als doctoraalstudenten bedanken voor het veldwerk, de verfrissende ideeën en het enthousiasme: bedankt, dziękuję, dankjewol! I am very grateful to Prof. dr. Wilfried Ernst, Prof. dr. Nick M. Waser and Prof. dr. Eddy van der Meijden for evaluating my thesis as members of the reviewing committee and for giving helpful comments. Ik wil graag Theo Peeters, Wouter van Steenis, Kobus Boeke, Kim Meijer, Riek van Noordwijk en wijlen Henny Wiering bedanken voor het determineren van vele insecten evenals de controles van determinaties, en Meike Bulten en Tineke Schwab voor het verzamelen van een deel van de veldgegevens. In de projectgroep Meta-Communities binnen het stimuleringsfonds Biodiversiteit van NWO-ALW zijn vele uitgebreide discussies gehouden, is goed commentaar op het werk geleverd en werden successen, vermaak en frustraties gedeeld: bedankt Jelmer Elzinga, Sonja Esch, Peter Klinkhamer, Arjen Biere, Carla Grashof-Bokdam, Jana Verboom, Sona Prakash, Arjan de Roos en Diana Prins (ook voor verzamelen van veldgegevens). Martina Stang, danke für den regen Informationsaustausch, die Anregungen zu neuen Ideen und die gemeinsamen Reisen! I would like to thank Juliet Osborne and Ingrid Williams for inviting Manja and me to Rothamsted Research (Harpenden, UK), where we enjoyed a very interesting and picturesque stay, and Amots Dafni for the invitation to Israel for the Pollination Techniques course on Har Meron and the pleasant stay in Haifa. Dick Visser wil ik hartelijk bedanken voor het opmaken van dit boekwerk, met een pluim voor

de nuchtere houding jegens de stuntelige promovendus. Joop Smittenberg bedank ik voor de contacten met de provincie Drenthe, de gastvrijheid en het doorgeven van boodschappen en papieren. Harmen Kraai, bedankt voor de assistentie bij de veldexperimenten, je liefde en het kunnen uithouden met een promovendus. Alma de Groot en Sandra van der Graaf, hartelijk dank voor jullie ondersteuning als paranimfen. SCAPE (Scandinavian Association of Pollination Ecologists): I loved the well organised meetings at remote locations, including discussions, sauna, fun, games and music. De promovendi van AIO+ wil ik graag bedanken voor de leuke discussiedagen. Staatsbosbeheer (SBB) ben ik zeer erkentelijk voor het verlenen van een vergunning voor veldwerk in het reservaat de Elperstroom. Ik heb de tijd bij Plantenoecologie / Community and Conservation Ecology erg genoten: het werk, lab, de gezelligheid, labuitjes, wederzijdse hulp, kamergenoten, etentjes, discussies, secretariaat, beantwoorden van vragen etc. Hierbij van mij een groots applaus voor de groep, inclusief alle tijdelijke en regelmatige gasten en studenten. Thank you PLOEC / COCON! Bedankt ook andere medewerkers op het Biologisch Centrum, vooral van de scretariaten van CEES en Functionele Ecologie. NWO-ALW heeft dit project gefinancierd via het Stimuleringsfonds Biodiversiteit, waarvoor ik zeer erkentelijk ben.



Curriculum vitae

I was born on February 4, 1974 in Zeven, Lower Saxony, Germany, in a family with a brother and a sister. Having a Dutch mother and a German father, I was raised bilingually in German and Dutch. In Zeven I attended four years of primary school (Grundschule Scheeßeler Straße) and two years of intermediate school (Orientierungsstufe Zeven). In 1986 I moved to Vaassen, the Netherlands. There I started secondary school at the Keienbrink M.A.V.O. and after one year I continued at the Koninklijke Scholengemeenschap in Apeldoorn. In 1993 I finished secondary school and started to study biology at the University of Groningen, specialising in Ecology. During my studies I did three MSc-projects: effects of herbivory on vegetation succession in coastal sand dunes on the Dutch island of Schiermonnikoog (supervised by dr. Adriaan van der Veen), energetics and chick-raising of Sandwich terns (Sterna sandvicensis) on the Danish island of Hirsholm (supervised by drs. Erwin Stienen, Alterra Wageningen), and restoration processes in calcareous grasslands on the Swedish island of Öland (supervised by Prof. dr. Jan P. Bakker, University of Groningen, and dr. Ejvind Rosén, Uppsala University). During the last project I stayed in Sweden for half a year. In addition, I did three weeks of fieldwork near Oxford, UK in 1999 (supervised by dr. Alison W. McDonald, University of Oxford). I graduated with an M.Sc in Ecology in 2000. Between the age of 14 and 25 I was an active member in the Nederlandse Jeugdbond voor Natuurstudie (NJN), a Dutch nature organisation for youth aged 12-25. I attended and organised many excursions and camps and was member of several commissions. During my studies I was also an active member in the Studenten Milieuoverleg Groningen (SMOG), a university environmental platform, organising debates, negotiations and actions. Between 2000 and 2005 I did a PhD at the University of Groningen about the importance of biodiversity for pollination in fragmented agricultural landscapes, resulting in the present thesis. In 2005 I moved to Hilversum, the Netherlands, where I live with my partner Harmen. Currently I have a position as translator at the translating agency Language Unlimited, Utrecht.