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## Effects of habitat isolation on pollinator communities and seed set

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**Abstract** Destruction and fragmentation of natural habitats is the major reason for the decreasing biodiversity in the agricultural landscape. Loss of populations may negatively affect biotic interactions and ecosystem stability. Here we tested the hypothesis that habitat fragmentation affects bee populations and thereby disrupts plant-pollinator interactions. We experimentally established small “habitat islands” of two self-incompatible, annual crucifers on eight calcareous grasslands and in the intensively managed agricultural landscape at increasing distances (up to 1000 m) from these species-rich grasslands to measure effects of isolation on both pollinator guilds and seed set, independently from patch size and density, resource availability and genetic erosion of plant populations. Each habitat island consisted of four pots each with one plant of mustard (*Sinapis arvensis*) and radish (*Raphanus sativus*). Increasing isolation of the small habitat islands resulted in both decreased abundance and species richness of flower-visiting bees (Hymenoptera: Apoidea). Mean body size of flower-visiting wild bees was larger on isolated than on nonisolated habitat islands emphasizing the positive correlation of body size and foraging distance. Abundance of flower-visiting honeybees depended on the distance from the nearest apiary. Abundance of other flower visitors such as hover flies did not change with increasing isolation. Number of seeds per fruit and per plant decreased significantly with increasing distance from the nearest grassland for both mustard and radish. Mean seed set per plant was halved at a distance of approximately 1000 m for mustard and at 250 m for radish. In accordance with expectations, seed set per plant was positively correlated with the number of flower-visiting bees. We found no evidence for resource limitation in the case of mustard and only marginal effects for radish. We conclude that habitat connectivity is essential to maintain not only abundant and diverse

bee communities, but also plant-pollinator interactions in economically important crops and endangered wild plants.

**Key words** Habitat fragmentation · Pollination · Resource limitation · Plant reproduction · Foraging radius

### Introduction

Destruction and fragmentation of habitats is the major reason for the worldwide decrease in biodiversity (Quinn and Harrison 1988). In man-made landscapes, agricultural activities are the most frequent cause of species loss and, in addition to these deterministic causes of extinction, reductions in area and increases in isolation of semi-natural habitats lead to further stochastic species losses (Saunders et al. 1991; Rosenzweig 1995).

Loss of biodiversity may result in the loss of stability and functioning of ecosystems (Lawton 1994; Naeem et al. 1995). Recent papers emphasize the importance of analyzing not only patterns of diversity but also the biotic interactions of the organism involved (LaSalle and Gauld 1993; Mills et al. 1993; Rathcke and Jules 1993; Matthies et al. 1995; Didham et al. 1996).

Habitat fragmentation may reduce species richness and abundance of pollinator guilds, change the foraging behavior of flower-visiting insects, disrupt plant-pollinator interactions, and reduce seed set and gene flow of isolated plant populations (Jennersten 1988; Lamont et al. 1993; Rathcke and Jules 1993; Aizen and Feinsinger 1994a, 1994b; Matthies et al. 1995; Didham et al. 1996).

Field studies are complicated by the fact that increasing fragmentation or isolation of habitats changes not only pollinator diversity but also other factors possibly influencing seed set like patch size and density of flowering plants, occurrence of competing alternative flowers (Klinkhamer and de Jong 1990; Jennersten and Nilsson 1993; Kunin 1993; Conner and Rush 1996), genetic variability, (Olesen and Jain 1994; Oostermeijer et al. 1994;

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Van Treuren et al. 1994; Westerbergh and Saura 1994; Matthies et al. 1995) and abundance of herbivores (Kruess and Tschardtke 1994; Strauss et al. 1996). Furthermore, seed set may be resource limited, but not pollinator limited, and therefore reductions in pollinator abundance would be less important (Bierzuchudek 1981; Horvitz and Schemske 1988; Burd 1994).

Bees (Hymenoptera: Apoidea) are one of the most important groups of pollinators, especially in temperate regions (LaSalle and Gauld 1993). In the agricultural landscape, maintenance of beneficial interactions such as crop pollination is a basis of sustainable agriculture. The diversity and geographical range of many wild (mostly solitary) bees has decreased in recent decades (Westrich 1989; Osborne et al. 1991; O'Toole 1993). Similarly, beekeeping is declining due to new disease and lower economic significance (Bienefeld 1996; Williams et al. 1991).

Here we tested the hypothesis that habitat isolation not only negatively affects bee diversity but also seed set of self-incompatible plants. To rule out alternative hypotheses, an experimental approach was used. We established small patches of two self-incompatible, annual plants at increasing distances from species-rich semi-natural grasslands to measure effects of isolation on both pollinator guilds and seed set independently of patch size and density, resource availability, and genetic erosion of plant populations.

## Materials and methods

### Study area and experimental design

The study was carried out in the "Leinebergland" near Göttingen, Germany, in 1995. The average annual temperature in this region is 8–8.5°C; the average annual rainfall is 600–700 mm (Hövmann 1963). The study area is an intensively managed agricultural landscape with islands of semi-natural, calcareous grasslands, mostly situated on the south or west slopes of hills. The grasslands developed by extensive grazing over a long period of time (at least decades). Today these habitats are characterized by a diverse vegetation with many endangered plant species and species-rich bee communities (Steffan-Dewenter 1998). Many are nature reserves.

We selected eight grasslands with a mean size of 4.3 ha as islands within an intensively managed surrounding area. The minimum distance between these grasslands was 2 km and the maximum distance 24 km. On each of the eight calcareous grasslands and at four distance classes (50–100 m, 100–300 m, 300–500 m, 500–1000 m) from these source habitats, we established small plant populations, here called "habitat islands." Each habitat island consisted of four pots 1 m apart, each planted with one plant of mustard (*Sinapis arvensis*) and one plant of radish (*Raphanus sativus*). The plants were fenced in with wire to prevent damage by herbivorous mammals. In total, we created 40 habitat islands with a total 160 pots. The direction of the eight isolation gradients, each consisting of five habitat islands, was randomized. The experimental plots of the four distance classes were placed on old and grassy field margins adjacent to farm tracks. Surrounding fields were dominated by wind-pollinated cereals, oil seed rape (not in blossom) and intensively managed meadows with low attractiveness for flower-visiting insects. Isolation of the habitat islands was determined by the distance from the nearest grassland. Additionally, we measured the distance from the nearest apiary which was slightly correlated with the distance from the nearest grassland ( $r^2=0.11$ ,  $P=0.04$ ).

### Experimental plants

Seeds of mustard (*S. arvensis*) and radish (*R. sativus*) were sown on 3 April and 18 April 1995, respectively, in a glasshouse. Young seedlings were separately transplanted into small pots. Between 15–19 May, one plant each of mustard and radish were planted into compartments (3.5 l) within larger pots (8.5 l, diameter 24 cm) using commercial garden soil (Einheitserde Typ T) with standardized high levels of nutrients. The lower part of the pots was used as a water supply and was connected with wicks (diameter 8 mm) to the upper soil compartment.

Potted plants were put in the field between 22–27 May to establish the experimental habitat islands. Plants were watered every 5–10 days depending on the weather conditions.

Both experimental plant species are known to be self-incompatible (Young and Stanton 1990; Kunin 1993). To test for the degree of pollinator dependence, branches of two additional mustard plants on each of six calcareous grasslands were isolated with gauze bags before flowering (Dafni 1992). Fruit set of bagged flowers was only 9% compared to about 90% in unbagged flowers. Mean number of seeds per fruit was  $1.6\pm 1.1$  in bagged versus  $9.6\pm 1.4$  in unbagged flowers ( $F=316$ ,  $n=52$ ,  $P<0.001$ ).

### Observation of pollinator visitation

Observations of flower-visiting insects were made between 19 June and 6 July 1995. Each plant species was observed for 15 min per habitat island to measure abundance and species composition of floral visitors. Bees and hover flies were identified to the species level, or at least to genus. The remaining flower visitors were separated into genera. Due to the short flowering time of radish, only 14 habitat islands could be observed. After the end of each 15-min observation period, wild bees were caught by sweeping for species determination.

### Reproductive success

Experimental plants were collected between 24–31 July (mustard) and 2–4 August (radish). To analyze seed set per fruit, 30 fruits of mustard and 10 fruits of radish were taken randomly from each plant. All remaining fruits per plant were taken together. Unfortunately, the mustard plants in six, and the radish plants in five habitat islands were partly damaged by deer. Therefore, whole-plant reproductive success could only be statistically analyzed with data of 34 habitat islands for mustard and 35 for radish. In addition, for radish, we counted the total number of flowers which did not develop into fruits to calculate the fruit set per plant.

Reproductive success was measured using the number of seeds per fruit, the seed weight per fruit, the number of fruits per plant, the number of seeds per plant, and the seed weight per plant.

### Statistical analyses

Statistical analyses of the data were performed using the software Statgraphics plus for Windows 2.1 (Statgraphics 1995). If necessary, logarithmic-or square-root-transformed variables were used to achieve normal distribution. Arcsine transformation ( $\arcsin\sqrt{p}$  where  $p$  is a proportion) was used for percentages (Sokal and Rohlf 1995). Stepwise multiple linear-regression analyses with forward selection were carried out to show the possible joint effects of independent variables. Analyses of variance (ANOVAs) were used to test for differences between two or more groups. Group differences were established using the Scheffe test at the 95% level. Arithmetic means $\pm$ 1 SD are given in the text.

**Table 1** Flower-visiting insects on mustard (*Sinapis arvensis*) and the relationships to two habitat characteristics (*DG* distance from the nearest grassland, *DA* distance from the nearest apiary). Results of stepwise multiple regression analyses are given

Species group	Number of individuals	Regression model	$r^2$	$P$
All bees (Hymenoptera: Apoidea)	179	$Y=7.98-0.25\sqrt{DG}$	0.28	<0.001
Solitary bees (Apoidea except <i>Apis</i> and <i>Bombus</i> )	85	$Y=2.56-0.83\sqrt{DG}+0.76\sqrt{DA}$	0.40	<0.001
Bumblebees ( <i>Bombus</i> spp.)	33	$Y=1.06-0.0008\sqrt{DG}$	0.08	0.069
Honeybees ( <i>Apis mellifera</i> )	61	$Y=3.52-0.07\sqrt{DA}$	0.19	0.005
Parasitoids (Hymenoptera parasitica)	474	Not significant	–	–
Ants (Formicoidea)	227	$Y=12.9-0.52\sqrt{DG}$	0.10	0.05
Cephalidae	38	Not significant	–	–
Beetles (Coleoptera)	518	$Y=6.31+0.45\sqrt{DG}$	0.11	0.04
<i>Meligethes</i> spp.	508	$Y=6.18+0.44\sqrt{DG}$	0.11	0.04
Flies (Diptera)	288	Not significant	–	–
Hover flies (Syrphidae)	70	Not significant	–	–
Bugs (Heteroptera)	18	Not significant	–	–
Butterflies (Lepidoptera)	3	Not significant	–	–
All flower visitors	1745	Not significant	–	–

## Results

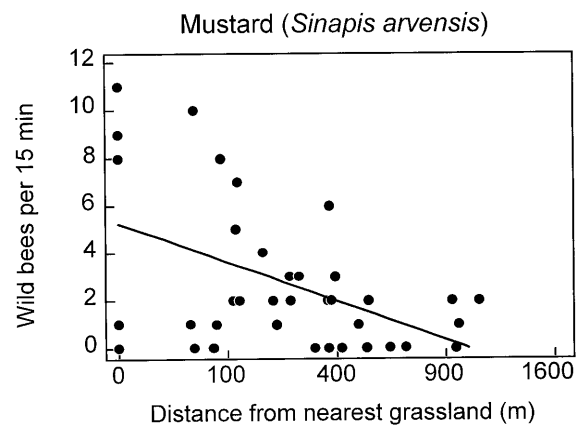
### Flower-visiting insects

Altogether, 1745 flower-visiting insects of five orders were observed on mustard. The most abundant groups were beetles (especially *Meligethes* spp.), parasitic Hymenoptera and flies (Table 1).

The most important group of pollinators, the bees, were represented with 179 individuals of solitary bees (Apoidea except *Apis* and *Bombus*), bumblebees (*Bombus* spp.) and honeybees (*Apis mellifera*) (Table 1). In total, 23 wild bee species (Apoidea except *Apis*) of seven genera were recognized. Abundance and species richness of flower-visiting wild bees were highly correlated ( $r^2=0.92$ ,  $n=40$ ,  $P<0.001$ ).

In stepwise multiple regression analyses we evaluated the importance of two habitat characteristics (distance from the nearest calcareous grassland and distance from the nearest apiary) for the distribution of the observed insect groups. The abundance of flower-visiting wild bees declined significantly with increasing distance from the nearest calcareous grassland (Fig. 1). Similarly, the species richness of flower-visiting wild bees decreased with increasing isolation distance ( $Y=3.02-0.08\sqrt{X}$ ,  $F=11.09$ ,  $r^2=0.23$ ,  $n=40$ ,  $P=0.002$ ). Additionally, 15% of the variance of wild bee abundance could be explained in a multiple model by a positive correlation with the distance from the nearest apiary (Table 1), possibly indicating exploitative competition by honeybees. The number of flower-visiting bumblebees only declined weakly with increasing distance from the nearest grassland. The abundance of flower-visiting honeybees on mustard declined with increasing distance from the nearest apiary (Table 1), and less significantly with increasing distance from the nearest grassland ( $F=4.99$ ,  $r^2=0.12$ ,  $P=0.03$ ).

In contrast, the abundance of the Coleoptera, dominated by *Meligethes* spp., increased slightly with dis-



**Fig. 1** Relationship between the number of flower-visiting wild bees on mustard (*Sinapis arvensis*) and the distance from the nearest calcareous grassland:  $Y=5.52-0.16\sqrt{X}$ ,  $F=12.6$ ,  $r^2=0.25$ ,  $n=40$ ,  $P=0.001$ . Note square root scale for distance

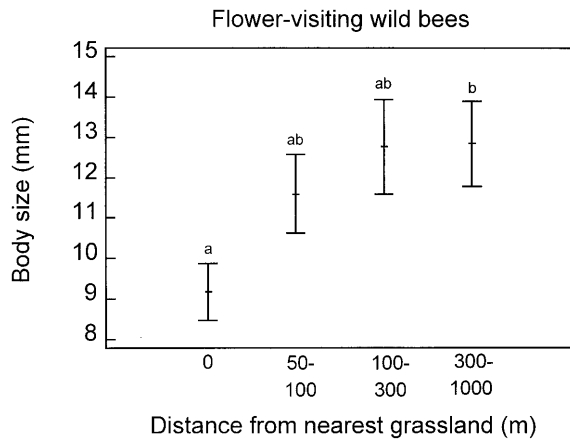
tance from the nearest grassland (Table 1). The remaining groups – flies, hover flies, bugs, Cephidae, and butterflies – as well as the sum of all flower visitors did not show a spatial pattern of distribution (Table 1).

Flower visitors on radish were analyzed in less detail because only 14 habitat islands could be observed. Altogether we recorded 45 solitary bees, but no honeybees and no bumblebees. The abundance of flower-visiting bees on radish declined significantly with increasing distance from the nearest semi-natural grassland ( $Y=6.08-0.21\sqrt{X}$ ,  $r^2=0.29$ ,  $n=14$ ,  $P=0.048$ ).

### Body size

We tested the hypothesis that more isolated habitat islands were visited by larger bees with better flight capabilities. The mean body size of flower-visiting bees was significantly smaller on habitat islands placed on the

grasslands than on habitat islands at a distance of 300–1000 m from the nearest grassland (Fig. 2). Furthermore, we found that the mean body size of flower-visiting bees on mustard was significantly larger than on radish ( $8.5 \pm 3.3$  versus  $4.8 \pm 0.1$  mm,  $F=54.1$ ,  $n=163$ ,  $P<0.001$ ).



**Fig. 2** Mean body size of flower-visiting wild bees on mustard (*S. arvensis*) and radish (*Raphanus sativus*) on the grasslands (0 m) and in three distance classes:  $F=4.09$ ,  $P=0.008$ ,  $n=163$ . The sum of observations of flower-visiting bees for the following classes was: 0 m,  $n=70$ ; 50–100 m,  $n=37$ ; 100–300 m,  $n=25$ ; 300–1000 m,  $n=31$ . Arithmetic means and pooled standard errors are given. The same letters indicate homogenous groups at the 95% level (Scheffe test)

## Seed set

Reproductive success was measured twice: first at the level of fruits and second at the level of the whole plant. We analyzed the significance of habitat and flower visitor parameters in two separate multiple regression analyses (Tables 2, 3).

### *S. arvensis*

The number of seeds per fruit declined slightly but significantly from 9.7 seeds per fruit on the grassland to 8.1 seeds per fruit 1000 m from the grasslands (Table 2). Stepwise multiple-regression analyses with the abundance of different groups of flower visitors as independent variables resulted in a multiple model with two contrary effects: number of seeds per fruit increased with increasing abundance of flower-visiting bees and decreased with increasing number of pollen beetles (*Meligethes* spp.) (Table 3).

The mean number of fruits per plant declined with increasing distance from the nearest grassland from 521 on grasslands to 313 at 1000 m (Table 2). Analyses of flower visitors showed a significant correlation between the mean number of fruits per plant and the number of flower-visiting bees per habitat island (Table 3).

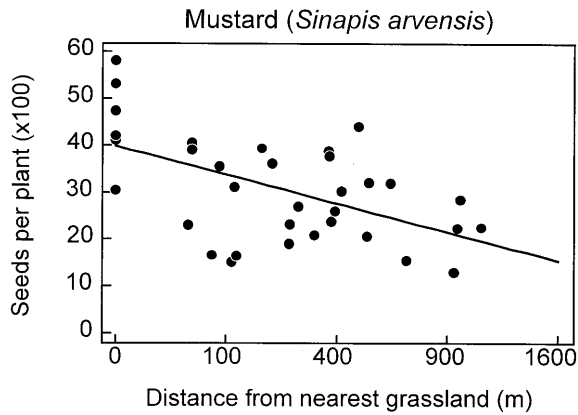
The number of seeds per plant, which reflects both the number of seeds per fruit and the number of fruits

**Table 2** Reproductive success of mustard and radish in relationship to two habitat parameters (DG distance from the nearest grassland, DA distance from the nearest apiary). Results of stepwise multiple regression analyses are given

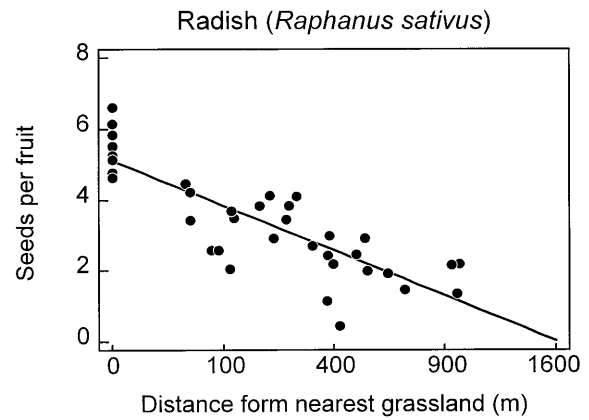
Parameters of reproductive success	Regression model	$r^2$	$n$	$P$
<b>Mustard (<i>Sinapis arvensis</i>)</b>				
Number of seeds per fruit	$Y=9.66-0.05\sqrt{DG}$	0.12	40	0.029
Number of fruits per plant	$Y=551.2-6.56\sqrt{DG}$	0.29	34	0.001
Number of seeds per plant	$Y=3976-60.8\sqrt{DG}$	0.28	34	0.001
Seed weight per plant (g)	$Y=6.82-0.10\sqrt{DG}$	0.29	34	0.001
<b>Radish (<i>Raphanus sativus</i>)</b>				
Number of seeds per fruit	$Y=5.1-0.13\sqrt{DG}$	0.69	35	<0.001
Number of fruits per plant	$Y=59.5-0.44\sqrt{DA}-0.58\sqrt{DG}$	0.29	35	0.004
Number of seeds per plant	$Y=133.3-4.21\sqrt{DG}$	0.46	35	<0.001
Fruit set per plant (%)	$Y=60.0-0.98\sqrt{DG}$	0.67	35	<0.001
Seed weight per plant (g)	$Y=0.98-0.03\sqrt{DG}$	0.40	35	<0.001

**Table 3** Reproductive success of mustard and radish in relation to the abundance of three independent flower visitor groups: B bees, PB pollen beetles, and syrphid flies for which no significant model was found. For radish, the number of bees was the only factor. Results of stepwise multiple regression analyses are given

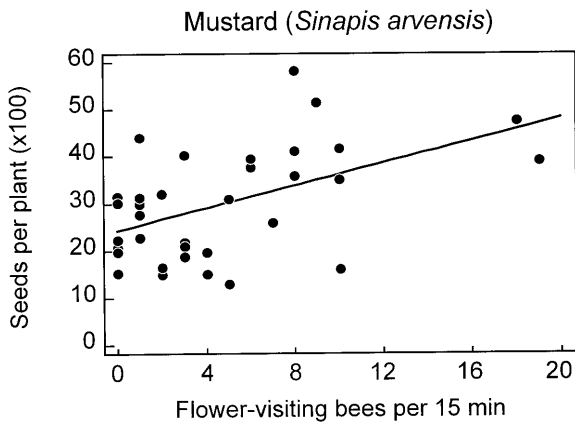
Parameters of reproductive success	Regression model	$r^2$	$n$	$P$
<b>Mustard (<i>Sinapis arvensis</i>)</b>				
Number of seeds per fruit	$Y=9.44-0.033PB+0.21B$	0.27	40	0.003
Number of fruits per plant	$Y=404.1+10.34B$	0.17	34	0.017
Number of seeds per plant	$Y=2429.4+119.1B$	0.24	34	0.003
Seed weight per plant (g)	$Y=4.28+0.18B$	0.20	34	0.008
<b>Radish (<i>Raphanus sativus</i>)</b>				
Number of seeds per fruit	$Y=2.74+0.21B$	0.49	14	0.005
Number of fruits per plant	Not significant			
Number of seeds per plant	$Y=21.8+2.1B$	0.40	14	0.016
Fruit set per plant (%)	$Y=42.0+1.54B$	0.31	14	0.036
Seed weight per plant (g)	$Y=0.58+0.05B$	0.42	14	0.023



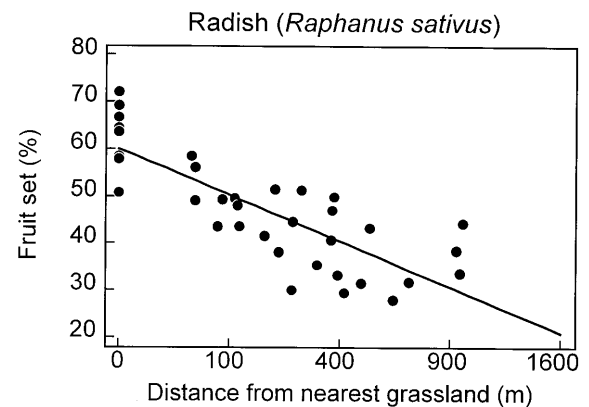
**Fig. 3** Relationship between the number of seeds per plant of mustard (*S. arvensis*) and the distance from the nearest calcareous grassland:  $Y=3976.7-60.8\sqrt{X}$ ,  $F=12.9$ ,  $r^2=0.28$ ,  $P=0.001$ ,  $n=34$ . Note square root scale for distance



**Fig. 5** Relationship between number of seeds per fruit of radish (*R. sativus*) and the distance from the nearest calcareous grassland:  $Y=5.1-0.13\sqrt{X}$ ,  $F=76.6$ ,  $r^2=0.69$ ,  $P<0.001$ ,  $n=35$ . Note square root scale for distance



**Fig. 4** Relationship between the mean number of seeds per plant of mustard (*S. arvensis*) and the abundance of flower-visiting bees per 15 min:  $Y=2546.4+116.3X$ ,  $F=10.36$ ,  $r^2=0.23$ ,  $P=0.003$ ,  $n=34$



**Fig. 6** Relationship between the proportion of radish flowers (*R. sativus*) which developed into fruits (percentages of fruit set are arcsine transformed) and the distance from the nearest calcareous grassland:  $Y=60.0-0.98\sqrt{X}$ ,  $F=59.4$ ,  $r^2=0.64$ ,  $n=35$ ,  $P<0.001$ . Note square root scale for distance

per plant, represents the best measure of reproductive success of an annual plant. It declined significantly with increasing distance from the nearest grassland (Fig. 3). Mean number of seeds per plant was approximately halved at a distance of 1000 m. Again, the number of flower-visiting bees was the best of all characteristics of flower visitation in predicting the number of seeds per plant (Fig. 4). If we separated all flower-visiting bees into honeybees and wild bees, the abundance of wild bees was the better predictor variable ( $r^2=0.18$ ,  $P=0.014$  against  $r^2=0.16$ ,  $P=0.019$ ).

The number of seeds per plant and the seed weight per plant were highly correlated ( $r^2=0.94$ ,  $n=34$ ,  $P<0.001$ ). Resource limitation of seed set may result in lower seed weight per seed with increasing number of seeds per plant. Therefore, we analyzed the relationship between the mean weight per seed (seed weight per plant/number of seeds per plant) and the number of seeds per plant, but this was not significant ( $r^2=0.05$ ,  $n=34$ ,  $P=0.22$ ).

#### *R. sativus*

Overall, the results found for radish were similar to those in mustard. The mean number of seeds per fruit declined more markedly from 5.1 to 2.3 seeds per fruit at a distance of 500 m from the grasslands (Fig. 5). The number of fruits per plant decreased with both the distance from the nearest grassland and the distance from the nearest apiary. The combination of these effects, i.e., reduced number of both fruits per plant and seeds per fruit, was to approximately halve the number of seeds per plant at a distance of 260 m from the nearest grassland (Table 2).

Additionally, we measured the fruit/flower ratio, i.e., the proportion of flowers that developed into fruits. It declined from 60% on the grasslands to 31% at 500 m from the nearest grassland (Fig. 6). The mean number of flowers per plant did not correlate with the distance from the nearest grasslands ( $r=+0.28$ ,  $n=35$ ,  $P=0.11$ ). Therefore, we can exclude effects of plant size on this result.

The parameters of radish reproductive success, i.e., number of seeds per fruit, number of seeds per plant, and fruit set per plant were positively correlated with the number of flower-visiting bees (Table 3).

Total seed weight and number of seeds per plant were highly correlated ( $r^2=0.895$ ,  $n=35$ ,  $P<0.001$ ), but we found a negative correlation between the mean weight per seed and the number of seeds per plant ( $Y=0.01-10^{-5}\times 1.46X$ ,  $r^2=0.20$ ,  $P=0.008$ ,  $n=35$ ).

## Discussion

Our study confirms two hypotheses: first, isolation from existing habitats diminishes abundance and species richness of bees, the most important pollinators; second, increasing isolation of the experimentally established habitat islands resulted in decreased seed set. Thus habitat isolation appeared to affect plant-pollinator interactions negatively by limiting the number of available pollinators and reducing the number of seeds per plant and, thereby, the potential reproductive success of the plants studied.

### Changes in pollinator communities

With increasing isolation of the habitat islands, the composition of flower visitors changed, but the total number of observed insects did not. The most important change was the reduction in wild bee visitation rates, obviously caused by the lack of favorable nesting sites and food supply in the intensively managed agricultural landscape (Westrich 1996). Wild bee populations are presumably mainly limited to the about 5% seminatural habitats that still exist in the study area. Distribution of honeybees depended primarily on the distance from the nearest apiary. However, this was slightly correlated with the distance from the nearest grasslands, possibly because many beekeepers prefer structurally rich areas and apiaries were located on four of the eight grasslands.

Similarly, abundance and species richness of euglossine bees declined in small forest fragments (Powell and Powell 1987). Jennersten (1988) found that abundance of flower-visiting insects on *Dianthus deltoides* was less in two fragmented populations than in a large continuous population. Habitat fragmentation may alter not only abundance and species richness of pollinators but also foraging behavior and body size (Rathcke and Jules 1993). With increasing distance from the nearest calcareous grassland, the abundance of flower-visiting wild bees on mustard and radish declined, and mean body size increased. This fits well with the expected positive relationship between foraging distance and body size (Gathmann et al. 1994; Wesslerling and Tschardtke 1995; van Nieuwstadt and Ruana Iraheta 1996). Additionally, this relationship may explain the fact that the abundance of flower-visiting bumblebees only declined slightly with increasing distance from natural habitats. Therefore,

bumblebees may be of special importance in maintaining pollinator services in the agricultural landscape (Corbet 1997).

### Changes in reproductive success

We used an experimental approach to separate expected pollinator limitation in more isolated plant populations from other factors possibly changing with habitat fragmentation. In both radish and mustard, the number of seeds per fruit, the number of fruits per plant, and the number of seeds per plant declined significantly with increasing isolation distance. Similarly, fruit set of radish decreased.

Other studies have shown that reproductive success measured as seed set can be pollinator or resource-limited (Bierzychudek 1981; De Jong and Klinkhamer 1989; Burd 1994; Johnson and Bond 1997). Reproductive success may also be affected by herbivorous insects, especially seed predators (Kruess and Tschardtke 1994; Didham et al. 1996) and changes in pollinator behavior, which is known to depend on patch size of flowering plants (Sowig 1989; Jennersten and Nilsson 1993; Kunin 1997), plant density (Kunin 1992), individual plant size (Klinkhamer and de Jong 1990; Ohara and Higashi 1994; Conner and Rush 1996) and background vegetation (Kunin 1993).

Habitat fragmentation possibly changes many of these factors, such as resource availability of plants, population size and density of flowering plants as well as abundance and species richness of pollinators. Furthermore, isolated plant populations may suffer from genetic erosion that diminishes reproductive success (Oostermeijer et al. 1994; Westerbergh and Saura 1994; Matthies et al. 1995).

In our study, seed set was positively correlated with the abundance of flower-visiting bees suggesting that reproductive success was pollinator limited. Evidence for resource limitation is less obvious. Only in radish did we find a slight tendency for increasing seed weight with decreasing number of seeds per plant. Furthermore, we used the same soil for all plants and, therefore, effects of resource limitation should have been the same at all habitat islands. Direct destruction of seeds by seed predators was only rarely observed. However, abundance of the pollen beetle, which is known to damage flower buds, slightly increased with increasing distance from the semi-natural grasslands. This may be explained by the release of herbivores from their natural enemies in more isolated habitats (Kruess and Tschardtke 1994; Thies et al. 1997). In multiple-regression analyses, seed set per fruit of mustard was negatively correlated with the abundance of pollen beetles and positively with the abundance of bees, whereas seed set per plant correlated only with the abundance of bees. Thus, there is little indication that seed set of the experimental plants was additionally reduced by herbivores.

Naturally developed small plant populations may have lower seed set due to genetic erosion resulting in

inbreeding depression. Using the same plant material for all habitat islands this can be excluded in our experiment.

Furthermore, identical patch size and density in all habitat islands allows us to exclude side effects due the preference by pollinators for larger or denser plant patches in less fragmented habitats.

The background of flowering plants was more diverse for the potted plants on the calcareous grasslands than for those in the surrounding agricultural landscape. The results of Kunin (1993) suggest that the “dissimilar background” on grasslands with many different flowers should affect seed set negatively compared to the background in the agricultural landscape with no or very few flowers. Accordingly, this background effect may compensate slightly for pollinator limitation in isolated habitats.

Seed set may also depend on the distance to the nearest plant population of the same species. Kunin (1992, 1993) showed that pollinator movements are mostly restricted to distances up to 20 m. Within this radius around our experimental habitat islands, we can exclude the occurrence of other populations of mustard or radish. Thus, pollen transfer was almost always within the exposed plants of the experimental habitat islands, regardless of the distance to the nearest calcareous grassland.

Seed set per plant was already halved at a distance of 250 m in radish, whereas seed set of mustard decreased less sharply and was halved at a distance of 1000 m. This may be related to differences in the mean body size of flower-visiting bees on radish and mustard and, therefore, a smaller foraging radius of flower visitors on radish.

Only a few other field studies have dealt with the effects of habitat fragmentation on plant-pollinator interactions. Jennersten (1988) found reduced seed set of two small isolated populations of *D. deltooides* in comparison to one large population in a semi-natural area. Aizen and Feinsinger (1994a) studied effects of tropical forest fragmentation on seed set of many different plant species. The mean seed set was reduced by about 20% in the most fragmented forest patches. Kunin (1992) showed that seed set of *Diplotaxis erucooides* decreased with increasing distance to the nearest conspecific neighbor. In small populations of *Banksia goodii* in Australia, seed set declined disproportionately (Lamont et al. 1993).

All these studies provide some evidence that habitat fragmentation affects seed set of small and isolated plant populations negatively, and thereby confirm the results of our study that clearly show both reduced abundance and species richness of bees and reduced seed set.

#### Possible consequences of lost plant-pollinator interactions

We expand the discussion to two more general ecological questions. (1) Is each plant species specialized for a few pollinators or is pollination possible by a wide vari-

ety of different species (Herrera 1988; Waser et al. 1997)? (2) Does the loss of species diversity definitely result in lost biotic interactions or are ecological systems redundant (Lawton 1994)?

Both plant species studied are expected to have a relatively unspecialized pollinator guild due to the easily accessible nectar and pollen (Heß 1983). This is confirmed by the great variety of different flower visitors on mustard in our study. The total number of flower visitors and, in particular, the number of syrphid flies known as important pollinators did not change, but seed set decreased with isolation distance. Accordingly, bees appeared to be the most efficient pollinators, whereas other pollinators were less effective, at least at the occurring densities. Experimental tests of different densities of syrphid flies in isolation cages with a self-sterile oilseed rape variety showed a weak pollination efficiency in comparison to a wild bee species (*Osmia rufa*) and honeybees (B. Grader and I. Steffan-Dewenter, unpublished results).

Seed set could be predicted by both abundance and diversity of bees, since abundance and species richness were closely correlated. We could not test the hypothesis that a mixture of species-specific patterns of temporal and spatial pollination activity significantly contribute to overall pollination success. So it remained unclear whether not only abundance (whatever species) but also diversity of bees mattered.

One might argue that honeybees and other long-distance foragers like social bumblebees are sufficient for pollination of all plant species. However, numbers of honeybee colonies are also decreasing due to new diseases and lower economic success (Bienenfeld 1996). In agricultural areas in particular, beekeeping is less attractive because only a few mass-flowering crops, for limited periods of time, are available (Williams et al. 1991). Furthermore, previous studies have shown that honeybees visit only some (20–30%) of all available plant species and therefore can be ruled out as possible pollinators for the remaining plant species (Buchmann 1996; Roubik 1996). Bumblebees are known to be important for the pollination of fragmented plant populations (Corbet 1997), but in our study, densities of observed bumblebees were low on mustard and absent on radish. This may be due to the bumblebee preference for deep flowers that contain a large standing crop of nectar (Corbet et al. 1995). Consequently, social bees obviously do not guarantee overall pollination, so that a diversity of solitary bees with their species-specific preferences in space and time are also necessary to maintain this important interaction type. The loss of plant-pollinator interactions is of great economic importance in that most (about 84%) of 264 crop species studied depend or benefit from insect visits for seed production (Williams 1996). Reduced reproductive success of allogamic plants might be expected to result in selective pressure to shift from self-sterility to self-fertility (Olesen and Jain 1994), but as yet we do not know how the loss of biotic interactions affects natural plant communities (Bond 1995).

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