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Experimental small-scale grassland fragmentation alters aphid population dynamics

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Theory predicts that at higher trophic levels species are especially vulnerable to habitat fragmentation due to small population size and dependence on particular prey species. Using aphids as model organism, we tested the hypothesis that herbivore abundance increases in fragmented habitats as a result of reduced predator and parasitoid densities. In a 3 year-study, we examined the population dynamics of aphids with respect to host plant abundance and ant nest density in experimentally fragmented calcareous grasslands at two sites in the northern Jura mountains. Fragments of different size (area: 20.25 m^2, 2.25 m^2 and 0.25 m^2) were isolated by a 5 m wide strip of frequently mown vegetation and corresponding control plots were situated in the adjacent undisturbed grassland. Aphid density was higher in fragments than in control plots. This was a combined result of a higher frequency of aphid-infested plants and larger aphid colonies in fragments than in control plots. Furthermore, a larger proportion of aphid colonies was ant-attended in fragments than in control plots. Aphid colonies were also more frequently visited by ants in fragments than in control plots in one of the 3 years. Parasitoid pressure on aphids was not influenced by the experimental fragmentation. Neither were aphid species richness and diversity affected by the fragmentation. Our study shows that even small-scale habitat fragmentation can have profound effects on the abundance of herbivorous insects. The effect on aphid density was consistent over 3 years and two sites with slightly different aphid communities.

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Habitat fragmentation is considered as a major threat to biodiversity (Saunders et al. 1991, Collinge 2000, Simberloff 2000). Fragmentation reduces the area suitable to the organisms and creates isolated subpopulations by disrupting the exchange of individuals and preventing gene flow (Lacy and Lindenmayer 1995). Fragmentation also influences interactions among species (Kruess and Tscharntke 1994, Groppe et al. 2001, Goverde et al. 2002) and ecological processes (Robinson et al. 1992).

Habitat fragmentation affects different species and different trophic groups to a different extent (Davies and Margules 1998, Debinski and Holt 2000, Zschokke et al. 2000). Theory predicts that at higher trophic levels species are especially vulnerable to fragmentation due to their small population size and dependence on particular prey species which itself may be affected by fragmentation (Holt 1996). There is some experimental evidence supporting this hypothesis. Aphids reached higher population densities in experimentally fragmented patches of goldenrod than in continuous habitat as a result of the predator's (ladybird beetles) delay to colonise the fragments (Kareiva 1984, 1987). Herbivorous insects occurred at increased densities in experimental patches of red clover as some of their predators and parasitoids failed to colonise the patches (Kruess and Tscharntke 1994).

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We examined the effect of experimental small-scale habitat fragmentation on the population dynamics of aphids in two calcareous grasslands. In contrast to other studies on the dynamics of one or a few herbivore species in patches of a single host plant we investigated a naturally diverse host plant-herbivore-predator system in a controlled field experiment.

Aphids are particularly useful model organisms because many species show high rates of population growth through parthenogenetic reproduction. Outbreaks may occur in the absence of predators and under favourable climatic conditions. Habitat fragmentation may affect aphid populations either directly or via their host plants, predators or mutualists. Firstly, migrating foundresses of aphid colonies may be differently attracted by small vegetation patches (fragments) and continuous habitats. The number of aphid colonies may increase in fragments when colonising females prefer small habitat patches. However, the contrary may occur when foundresses prefer continuous habitats. Secondly, fragmentation may affect the distribution and abundance of host plants (Robinson and Quinn 1988, Holt et al. 1995). As many aphid species depend on one or a few host species, the distribution of host plants will influence aphid species composition in fragments. Thirdly, plants growing along the edges of fragments are exposed to altered microclimatic conditions (Laurance and Yensen 1991). In addition, plants at the edges of fragments may benefit from reduced competition for light and nutrients. These factors may increase host plant quality which in turn enhances aphid population growth (Breton and Addicott 1992, Kindlmann and Dixon 1992, Stadler 1995). Indeed, several studies revealed positive effects of habitat edges on the dynamics of insect species (Roland and Kaupp 1995, Cappuccino and Martin 1997). Fourthly, many aphid species interact with ants. In our long-term experiment fragmentation affected the density and species composition of ants (B. Braschler, unpubl.). Fragmentation also influenced other food resources of ants. Thus, ant-tending intensity should increase and ant predation on attended aphids decrease when alternative sugar resources like extrafloral nectaries (Offenberg 2000, 2001, Sakata and Hashimoto 2000) or other homopteran colonies are in short supply (Cushman and Addicott 1989). Changes in ant species composition due to fragmentation could influence aphid populations as different ant species attended aphids in different ways (Addicott 1978, 1979). Changes in ant attendance may further affect parasitoid pressure on aphids (Völkl 1992). In general, ant-attended aphids benefit from an increased ant density. Finally, some predators and parasitoids may be absent in fragments and thus release herbivores from top-down control and potentially allow outbreaks (Kruess and Tscharntke 1994, 2000).

In the present paper we tested these hypotheses using data on aphid density and species composition, host plant abundance, interactions among aphids and attending ants and parasitoid pressure collected over 3 years in a controlled field experiment.

Material and methods

Study sites

The fragmentation experiment was carried out in two calcareous grasslands situated in the northern Swiss Jura mountains: in Nenzlingen (13 km S of Basel; 47° 34′ N, 7° 35′ E) and Vicques (26 km SSW of Basel). Originally covered by beech forest, these grasslands have been grazed by cattle for many centuries, leading to the characteristic vegetation of the *Teucrio-Mesobrometum* (Zoller 1947, Ellenberg 1986). A description of the sites is given in Zschokke et al. (2000).

Fragmentation experiment

The experimental fragmentation of the grasslands was created in spring 1993 by mowing the vegetation around the experimental fragments. One experimental unit, called block, contained one large (4.5×4.5 m), one medium (1.5×1.5 m) and two small (0.5×0.5 m) fragments, all of them separated by a 5 m wide strip of mown vegetation, as well as the corresponding control plots, which were mirror-symmetrically arranged and surrounded by undisturbed vegetation (Fig. 1). Within each block, the positions of the different sizes of fragment-control plot pairs as well as the control and fragment halves were randomised. The experimental set-up used in the present study consisted of 9 blocks (five in Nenzlingen and four in Vicques) with 36 fragments (9 large, 9 medium and 18 small) and 36 corresponding control plots. The distances between blocks within the sites ranged from 25 to 135 m. The blocks were part of larger study areas (1.5–2 ha) that were enclosed by fences to exclude large herbivores. The experimental fragmentation had been maintained since April 1993 by frequently (6–12 times per year) mowing the area between the fragments in the period from March to October. The entire experimental area was mown in late autumn every year to prevent succession (Kienzle 1979).

Field methods

Above-ground aphid density was assessed in the years 1997–1999 by recording the number of aphids on infested plants growing in fragments and control plots. Aphid density was calculated as number of aphids per 0.25 m^2. Aphid colony size was defined as number of aphids of the same species occurring on a single plant.

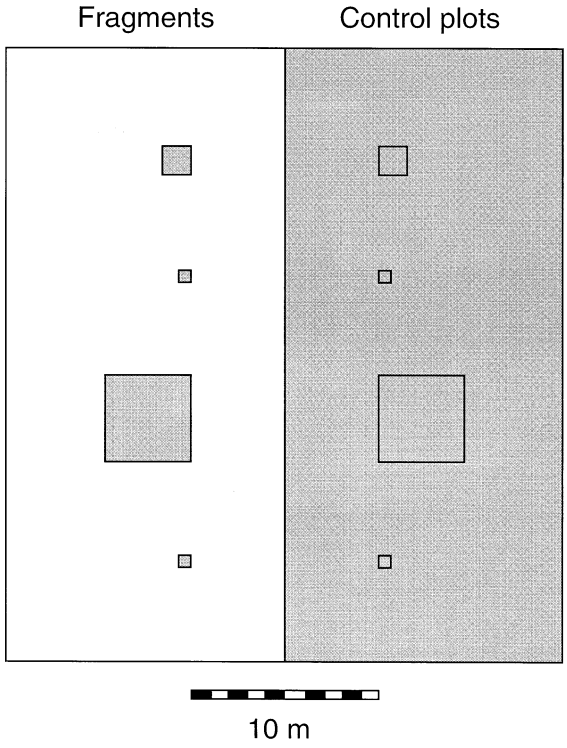

Fig. 1. Diagram of one block of the fragmentation experiment. A block contained two small (0.5×0.5 m), one medium (1.5×1.5 m) and one large (4.5×4.5 m) fragment and corresponding control plots. The isolation area between the fragments (shown in white) was frequently mown.

Most colonies were small enough to count all individuals. In large colonies (> 100 individuals) the area covered by aphids on a plant was determined and a subsample from a defined area was collected using a paintbrush. The number of individuals counted in the subsample was then extrapolated to the entire area occupied by the colony. The number of aphid mummies was also recorded as a measure of parasitoid pressure.

The species of aphid-infested plants were determined. The density of infested plants was calculated as number of infested plants per 0.25 m^2. To assess infestation rates, we recorded the numbers of both infested and non-infested plants in the three species most frequently infested by aphids and in another nine frequently infested species in 1999. The plant species were chosen according to the following four criteria: presence in both study sites, infestation with aphids in 1997 or 1998, easy to determine when not flowering, only one species per genus. Nomenclature of the plants follows Binz and Heitz (1990).

In 1997, aphid density was assessed in 32 fragments and 32 control plots (five blocks in Nenzlingen and three blocks in Vicques). In small patches (fragments and control plots) we determined aphid number on each host plant. In medium-sized patches we assessed aphid number in five subplots measuring 50×50 cm. One of these subplots was located in the centre of the patch and four at the edge. In large patches, aphid number was determined in twelve subplots: four in the centre and eight at the edge.

In 1998 and 1999, aphid density was assessed in 36 fragments and 36 control plots (five blocks in Nenzlingen and four blocks in Vicques). In small patches the aphids were recorded on each infested plant. In medium-sized patches we recorded aphids in three subplots (one in the centre and two at the edge). In large patches, aphid density was assessed in 27 subplots, which corresponded to one third of the entire area. Sixteen subplots were randomly chosen from the centre area and eleven at the edge.

Aphid density was recorded between 2 and 29 July 1997, 18 and 30 May 1998 and 1 and 24 July 1999. The order of field work followed the stage of the vegetation period: plots in Nenzlingen were studied before those in Vicques. The order in which fragments and control plots of the same block were studied was randomised.

The present study was part of a larger project to examine effects of grassland fragmentation on plants and different groups of invertebrates over several years. For this reason we used non-destructive sampling methods whenever possible. In 1998, however, up to 17 aphids (most frequently one to six individuals) were collected from each colony and preserved in 70% alcohol for later species identification. Whenever possible the majority of individuals of a colony was left on the plants to minimise the impact of sampling. On some plants only one or very few aphids were found. Some aphids dropped from the plant before sampling. Species identification was not always possible when only nymphs or alatae (winged aphids) were present. Thus, some colonies were not considered in a few analyses.

The aphids were mounted for species identification. This was done by first removing all embryos and then embedding the aphids into a mixture of polyvinylalcohol and lactophenol following the method of Heinze (1952). All aphids collected were identified by one of us (GL). Nomenclature of the aphids follows Remaudière and Remaudière (1997).

Ant-tending intensity was assessed as the number of ants that tended aphids when the colony was surveyed for the first time. Visitation rate was estimated as number of ants tending a colony within 2 min. Colonies not visited during 2 min. were classified as not visited. In the case of a misclassification this procedure makes our interpretations more conservative.

The density of ant nests was recorded in large experimental plots between 29 July and 10 September 1999 to examine whether differences in ant-tending intensity were a result of differential ant density. Nests were identified by carefully searching the plots for visual signs and following the workers from baits (plastic caps 2.9 cm in diameter filled with sucrose solution) back to

their nests. Fragments and control plots of the same block were examined on the same day to avoid any confounding influence of the season. Similarly, foraging activity of ants (number of ants recorded in 2 min on a bait, 1 h after it was offered) was recorded in large experimental plots in 1999. In each patch we placed 181 baits (sucrose solution baits as above). Ant species were identified on site, but in some cases a few specimens were removed for later identification in the lab using a binocular microscope. Nomenclature of the ants follows Bolton (1995).

Statistical analyses

A repeated measurement ANOVA (type III) was used to examine the effects of fragmentation, study site (Nenzlingen or Vicques), block (nested in site, random factor) and patch size (large, medium, small) on aphid density over all years. Due to missing data in four fragments and four control plots in 1997, only a reduced data set could be used for this analysis. An ANOVA (type III) was used to evaluate the data from each year separately. Using the same ANOVA model, fragmentation effects on colony size and density of aphid-infested plants were evaluated. To examine whether aphid density differed between the edge zone (a 50 cm wide strip along the edge) and the interior (core area) of fragments and control plots, an ANOVA with the additional factor 'edge' was used. Possible edge effects were only analysed in medium-sized and large plots (small plots consisted exclusively of edge zone). Unpaired t-tests were used to examine fragmentation effects on the densities of the two most abundant aphid species. As variances were unequal between groups approximate degrees of freedom were calculated following Satterthwaite (1946). Aphid densities of single species were calculated on the basis of plots occupied by the particular species (Appendix A).

Differences in infestation rate between fragments and control plots were examined in 12 plant species using Fisher's exact tests. As aphid species composition differed between study sites, data from each site were analysed separately. Similarly, the effect of fragmentation on the frequency of ant-attended aphid colonies and on parasitoid pressure were analysed using Fisher's exact tests. To eliminate confounding factors such as different weather conditions, paired t-tests were used to examine differences in ant nest density and forager activity between large fragments and corresponding control plots. Ant-tending intensity depended on aphid colony size. Differences in ant-tending intensity between fragments and control plots were examined by comparing the residuals of the logarithmic regression of ant-tending intensity $= 0.53\text{-}0.094 \times \log(\text{mean aphid colony size})$ using Mann-Whitney U-tests (data did not fit normal distributions).

Unpaired t-tests were used to analyse differences in aphid diversity (Shannon-Wiener diversity index) between large fragments and control plots. Statistical analyses were performed using SAS 6.08 (SAS Institute 1990) for linear models and StatView 5.0 (SAS Institute 1998) for Fisher's exact tests and correlations. Data on aphid colony size and ant density were log-transformed, those on aphid density and density of infested plants $\log(y+1)$. In the text and figures mean values are presented with 95% confidence intervals (note that backtransformed values have asymmetric error bars (Sokal and Rohlf 1995)).

Results

Effect on aphid density

The experimental fragmentation affected the number of aphids (Fig. 2, Table 1). Aphid density was higher in fragments than in control plots (repeated measurement ANOVA, $F_{1,44} = 12.71$, $p = 0.0009$). However, aphid density varied also among years (Fig. 2; repeated measurement ANOVA, $F_{2,88} = 60.43$, $p < 0.0001$) and between study sites ($F_{1,44} = 7.15$, $p = 0.0105$). Aphid density was higher in Nenzlingen than in Vicques in 1999. Aphid density was also affected by patch size ($F_{2,44} = 3.80$, $p = 0.0300$). However, this effect was differently pronounced in different years (Fig. 3, Table 1). Furthermore, there was a significant interaction between experimental fragmentation and patch size in 1999 (Table 1). No consistent edge effects on aphid density were found. In 1998, we determined all aphid

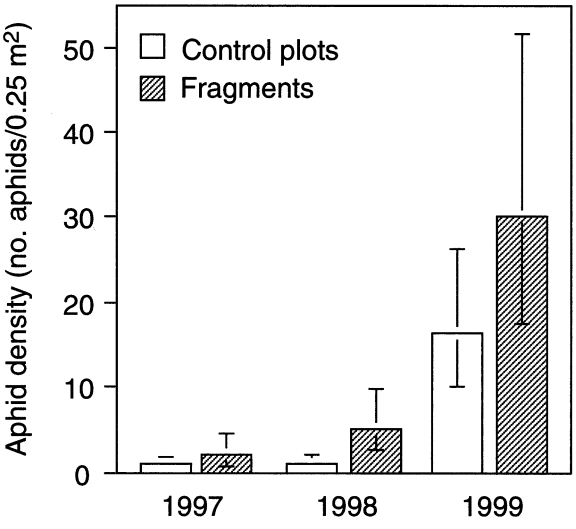

Fig. 2. Among-year variation in aphid density (number of individuals per 0.25 m^2, mean $\pm$ 95% confidence interval) in fragments and control plots. 32 fragments and 32 control plots were examined in 1997. In 1998 and 1999, 36 fragments and 36 control plots were examined. For statistical tests see Table 1.

Table 1. Summary of ANOVAs (type III) testing the effects of experimental fragmentation (T), study sites (S), block (B, nested in S) and patch size (P) on density of aphids. B(S) and the interaction T × B(S) were entered as random factors. In 1997 only 8 out of 9 blocks were examined.

Source	1997				1998				1999			
	df	MS	*F*	*p*	df	MS	*F*	*p*	df	MS	*F*	*p*
T	1	3.7712	3.1279	0.1105	1	25.6100	29.2567	0.0001	1	11.6695	6.0954	0.0334
S	1	9.8291	4.3564	0.0819	1	6.8154	3.4462	0.1058	1	7.0968	6.4919	0.0382
B(S)	6	2.2562	1.9279	0.2064	7	1.9777	2.5837	0.1035	7	1.0932	0.5725	0.7617
P	2	7.7633	5.2199	0.0092	2	8.4070	4.5091	0.0158	2	1.9174	0.9787	0.3829
T × B(S)	7	1.1744	0.7896	0.5998	8	0.7655	0.4106	0.9092	8	1.9095	0.9746	0.4665
T × P	2	0.4504	0.3029	0.7402	2	3.4066	1.8271	0.1715	2	7.5181	3.8374	0.0282
Error	44	1.4873			50	1.8645			50	1.9592		

species. Considering the density of single species, contrasting results were found for the two most abundant species. *Acyrthosiphon malvae poterii* Prior et Stroyan was more abundant in fragments than in control plots (aphids/0.25 m^2: fragments: 0.27 (0.06, 0.53); control plots: 0.02 (0.00, 0.04); $t = 2.48$, $df = 35.7$, $p = 0.0181$), while no difference in the density of *Aphis stachydis* Mordvilko between fragments and control plots was found (aphids/0.25 m^2: fragments: 0.18 (0.02, 0.36); control plots: 0.22 (-0.06, 0.57); $t = 0.22$, $df = 55.9$, $p = 0.83$). Species other than *A. malvae* contributing to the overall higher aphid density in fragments were: *Aphis craccivora* Koch, *Aphis euphorbiae* Kaltenbach, *Aphis helianthemi* Ferrari, *Myzus langei* (Börner) and *Aphis* sp. on *Origanum vulgare* L. These species were abundant in some fragments, but no individuals were found in control plots (Appendix A). Furthermore, *Macrosiphum rosae* (Linnaeus) mainly occurred in fragments. Some species represented by a few individuals were recorded either in fragments or in control plots (Appendix A).

The higher aphid density in fragments was mainly a result of a larger number of plants infested by aphids in fragments than in control plots (Fig. 4). Fragments contained a larger number of infested plants in all years, although in 1999 there was only a tendency (ANOVA, 1997: $F_{1,9.24} = 6.80$, $p = 0.0278$; 1998: $F_{1,10.11} = 11.82$, $p = 0.0063$; 1999: $F_{1,9.66} = 3.46$, $p = 0.0935$). A larger number of infested plants could result from a higher density of potential host plants or a higher rate of infestation. To distinguish between these two explanations, the infestation rate was measured in twelve plant species which were frequently infested by aphids in 1999. Considering data from both study sites separately, this would result in 24 different fragment-control plot comparisons. However, no individuals of the plant species *Lathyrus pratensis* L. were found in Vicques, reducing the total number of comparisons to 23. In five out of 23 fragment-control plot comparisons, potential host plants showed a higher infestation rate in fragments than in control plots, in a further comparison there was a tendency (Table 2). No plant species was significantly more often infested by aphids in control plots than in fragments after sequential Bonferroni correction. This indicates an enhanced infestation risk for some plants in fragments. Most plant species showed no difference in abundance between fragments and control plots. In only two comparisons a plant species was more abundant in fragments than in control plots (*Agrimonia eupatoria* L. and *Knautia arvensis* Duby, both in Vicques) and in another two comparisons a plant species was more abundant in control plots than fragments (*Betonica officinalis* L. in Vicques and *Salvia pratensis* L. in Nenzlingen) after sequential Bonferroni correction. However, in only one of these cases (*K. arvensis* in Vicques) the infestation rate differed between fragments and control plots. This indicates that the higher infestation rates of some plant species in fragments were not a result of differences in abundance.

Aphid colonies in fragments were larger than those in control plots in 1999 (mean number of individuals: fragments: 15.91 (10.69, 23.67), control plots: 10.12 (7.29, 14.04); ANOVA, $F_{1,9.55} = 6.63$, $p = 0.0287$), while in the other 2 years no significant difference was found (1997: $F_{1,13.50} = 1.31$, $p = 0.27$; 1998: $F_{1,16.77} = 0.01$, $p = 0.93$). Considering all years, large aphid colonies ($\geq$ 100 individuals) occurred more frequently in fragments (5.6% of colonies) than in control plots (1.9%; Fisher's exact test: $p = 0.0006$). The majority of species were represented by only one or a few individuals. This prevented any comparisons between fragments and control plots. In the two most abundant species, *Aphis stachydis* with ant-attendance and *Acyrtosiphon malvae poterii* without ant-attendance, no difference in colony size between fragments and control plots was found (mean number of individuals: *A. stachydis*: 7.89 (3.18, 19.61) vs 10.94 (2.46, 48.62), $t = 0.48$, $df = 13$, $p = 0.64$; *A. malvae*: 1.57 (1.14, 2.15) vs 1.78 (0.95, 3.34), $t = 0.46$, $df = 18$, $p = 0.65$).

Aphid species diversity

A total of 24 aphid species were recorded on 19 different plant species in 1998 (Appendix A). Most aphid species were rare; in 14 species only a single individual

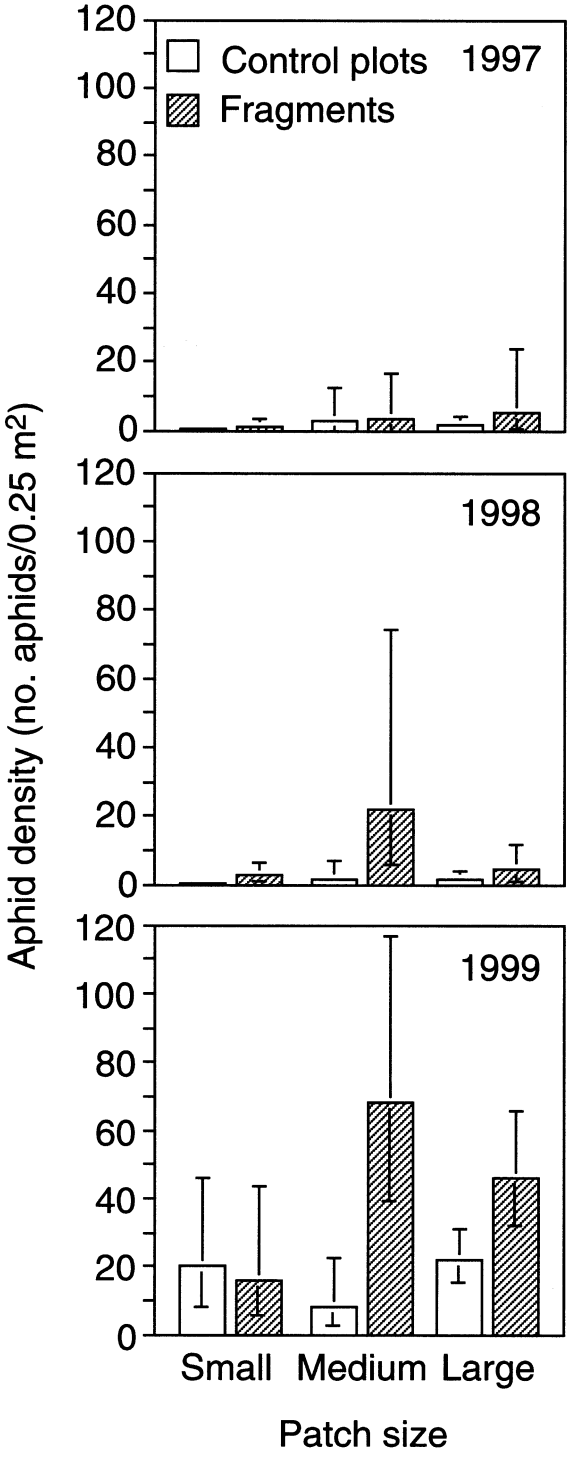

Fig. 3. Effect of patch size on the density of aphids (number of individuals per 0.25 m^2, mean $\pm$ 95% confidence interval) in 1997–1999. In 1997, we examined 16 small, 8 medium and 8 large fragments and control plots each. In 1998 and 1999, the sample size was 18 for small, 9 for medium and 9 for large fragments and control plots each. For statistical tests see Table 1.

or a single colony were found. The species composition differed between the two study sites. Six of the 24 species (25%) were found at both sites. Considering the diversity of plant species infested by aphids, further 13 plant species were infested by aphids in 1997 and 1999 (Appendix B).

Considering large plots, experimental habitat fragmentation did not affect aphid species richness (fragments: 4.11 (2.81, 5.41), control plots: 3.11 (1.70, 4.52), $t = 1.20$, $df = 16$, $p = 0.25$) and diversity (Shannon-Wiener index, fragments: 1.09 (0.90, 1.27), control plots: 0.92 (0.42, 1.41), $t = 0.74$, $df = 16$, $p = 0.47$).

Effects on aphid-ant interactions

The densities of ant nests and foragers were assessed in large fragments and the corresponding control plots in 1999. Fragments had a higher density of ant nests than control plots (number of nests per 0.25 m^2: fragments: 0.23 (0.20, 0.26), control plots: 0.17 (0.13, 0.22); paired $t = 2.62$, $df = 8$, $p = 0.0306$). Forager density (number of ants counted per bait in 2 min) was larger in fragments than in control plots (1.46 (0.99, 2.12) vs 0.61 (0.33, 1.12); paired $t = 3.73$, $df = 8$, $p = 0.0058$).

Most aphid species recorded were ant-attended. Exceptions were some rare species and *Acyrthosiphon malvae poterii* of which numerous colonies occurred at both study sites (species without ant-attendance accounted for 35% of colonies and 6% of individual aphids in 1998). The majority of ants that attended aphids belonged to *Lasius paralienus* Seifert. *L. paralienus* was also the most abundant ant species in

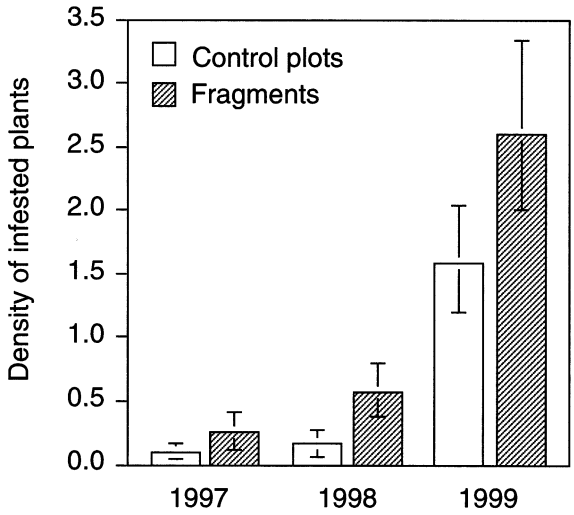

Fig. 4. Effect of habitat fragmentation on the density of plants infested by aphids (number of infested plants per 0.25 m^2, mean $\pm$ 95% confidence interval) in 1997–1999. 32 fragments and 32 control plots were examined in 1997. In 1998 and 1999, 36 fragments and 36 control plots were examined.

Table 2. Number of aphid-infested and aphid-free individuals in 12 plant species in fragments and control plots in Nenzlingen (N) and Vicques (V) in 1999. *Lathyrus pratensis* was not recorded in Vicques. * indicates a higher infestation rate in fragments than in control plots, $p<0.05$ after sequential Bonferroni correction, + indicates a trend for a difference ($p<0.1$).

Plant species	Study site	Fragments		Control plots		Fisher's exact test
		Number of plants		Number of plants		
		with aphids	without aphids	with aphids	without aphids	p
Agrimonia eupatoria L.	N	1	20	4	20	0.35
	V	12	59	2	27	0.34
Betonica officinalis L.	N	86	39	36	52	<0.0001*
	V	80	100	116	139	0.85
Centaurea jacea L.	N	0	2	0	6	>0.99
	V	4	37	9	43	0.37
Helianthemum nummularium Miller	N	3	10	2	15	0.63
	V	0	18	0	30	>0.99
Hypericum perforatum L.	N	8	8	9	16	0.52
	V	20	25	15	19	>0.99
Knautia arvensis Duby	N	49	22	27	46	0.0001*
	V	13	75	0	45	0.0045+
Lathyrus pratensis L.	N	22	3	11	0	0.54
Lotus corniculatus L.	N	25	33	13	20	0.83
	V	15	73	0	110	<0.0001*
Plantago media L.	N	4	11	3	13	0.69
	V	3	40	12	36	0.0249
Salvia pratensis L.	N	2	14	7	134	0.23
	V	0	7	0	17	>0.99
Sanguisorba minor Scop.	N	188	267	93	292	<0.0001*
	V	31	294	18	285	0.10
Scabiosa columbaria L.	N	7	3	1	17	0.0007*
	V	0	0	0	4	>0.99

the study sites (60–80% of ant nests recorded in the experimental plots and 97% of the aphid-attending ants). Other aphid-attending ants were in order of decreasing abundance: *Myrmica sabuleti* Meinert, *Myrmica scabrinodis* Nylander, *Myrmica schencki* Emery, *Lasius flavus* (Fabricius), *Formica pratensis* Retzius, *Formica rufibarbis* Fabricius, *Formica cunicularia* Latreille, *Lasius niger* (Linnaeus).

Considering data from 1997–1999, a larger proportion of the aphid colonies in fragments were attended by ants than in control plots (fragments: 41.4%, control plots: 32.4%; Fisher's exact test, $p=0.0009$). Ant-attended aphid colonies were larger than aphid colonies not visited by ants (mean number of individuals: 28.9 (23.7, 35.2) vs 4.7 (3.9, 5.7); $t=12.90$, $df=211$, $p=0.0001$). The larger proportion of ant-attended colonies may contribute to the higher aphid density in fragments than in control plots, though both ant-attended and unattended colonies were more numerous in fragments than in control plots (Fisher's exact test, $p<0.0001$ in both cases). Considering data collected in large plots in 1999, aphid density tended to be correlated with ant nest density in fragments ($r=0.60$, $n=9$, $p=0.0893$), but not in control plots ($r=-0.46$, $n=9$, $p=0.22$). In contrast, aphid density was not correlated with ant forager density in large experimental plots in 1999 (fragments: $r=0.34$, $n=9$, $p=0.39$, control plots: $r=-0.12$, $n=9$, $p=0.77$). In 1998, attended colonies were more intensively visited by ants in fragments than in control plots (Mann-Whitney U-test, $p=0.0477$). In 1997 and 1999, there was no difference in frequency of visiting ants between fragments and control plots (1997: $p=0.96$; 1999: $p=0.59$).

Parasitoid pressure

As a measure of parasitoid pressure we counted the number of aphid mummies. Mummified aphids were found both in fragments and control plots in 1997 and 1999. In 1998, no mummified aphids were found either in fragments or in control plots. The proportion of aphid colonies with mummies was higher in 1997 (23.8%) than in 1999 (4.1%; Fisher's exact test, $p<0.0001$). A total of 730 mummies were found in 1997 and 391 in 1999. Thus, the number of mummies was twice as large in 1997 than in 1999, even though the total number of recorded aphids was smaller in 1997 than in 1999 (3174 vs 22 773 individuals). However, the experimental habitat fragmentation neither affected the proportion of aphid colonies that were parasitised (considering data from 1997 and 1999, Fisher's exact test, $p>0.99$) nor the number of mummies (ANCOVAs with fragmentation as fixed factor and mean aphid colony size as covariate; 1997: $F_{1,3}=2.83$, $p=0.19$; 1999: $F_{1,13}=0.86$, $p=0.37$).

Discussion

The present study shows that the experimental habitat fragmentation caused an increased aphid density in fragments over 3 years at two sites with different aphid and plant species compositions. Thus, the fragmentation effect was temporarily and spatially replicated. The weather conditions may vary between sites and among years. However, the fragmentation effect was consistent despite variation in environmental conditions which influence local aphid density.

The experimental fragmentation resulted in a larger number of aphid colonies and a higher infestation rate of some host plants, but also in a trend towards larger colonies in fragments. Large aphid colonies can seriously harm their host plant (Dixon 1985). In our field experiment, most colonies were relatively small. However, sampling later in the year would have revealed larger colony sizes as some aphid species reach their peak colony size later in the season. The fragmentation seemed to promote aphid colony growth. Large aphid colonies were found more frequently in fragments than in control plots. Furthermore, mean aphid colony size was larger in fragments than in control plots in one year.

In most of the plant species examined only a minority of individuals were infested by aphids. However, more plants may have become infested later in the season. Alatae (= winged aphids) production can be caused by crowding (Robert 1987). Indeed, a larger proportion of colonies with alatae occurred in fragments than in control plots (B. Braschler, unpubl.). Thus, there were more alatae in fragments to colonise new plants. Furthermore, it has been shown that apterous (= unwinged) aphids may be able to colonise neighbouring plants, though the frequency with which apterae migrate to other plants differs among species (Edson 1985, Cappuccino 1987). In some species the apterae's tendency to migrate increases with crowding (Robert 1987). Combined, the higher proportion of alatae and the larger colony size in fragments may indicate a relatively high risk of future infestation for potential host plants that were not colonised at the sampling date.

In 1999, the final year of the aphid survey, no significant fragmentation effect on species richness and species composition of plants was found (H.-P. Rusterholz, unpubl.). In the plant species examined the infestation rate was not related to the relative abundance in the plots. Plant quality can promote population growth of aphids (Breton and Addicott 1992). We did not asses plant quality. However, Dolt (2001) found an increased above-ground plant biomass in fragments in the years 1996–1998. Furthermore, in fragments plant biomass tended to be larger in the edge zone than in the core area (Dolt 2001). Plant productivity may be an indirect indicator of plant quality as many aphids grow best under conditions of high nutrient transport (like in growing and wilting plants). Indeed, aphid density increased with plant biomass in experimental plots in 1997 (B. Braschler, unpubl.). However, our results showed no consistent edge effect on aphid density in fragments.

The experimental fragmentation did not reduce the parasitation rate of aphids. This contrasts predictions from theoretical models (Holt 1996) and findings from other fragmentation studies (Kruess and Tscharntke 1994, 2000). The isolation distance of fragments (5 m) used in our field experiment seems to be small for flying parasitoids. However, previous results showed that the abundance of foraging butterflies was negatively affected by this type of fragmentation (Zschokke et al. 2000). Furthermore, Goverde et al. (2002) found that the abundance of a bumblebee species was negatively affected by the fragmentation and that the bumblebee's foraging behaviour was changed in fragments. In another fragmentation experiment, ladybird beetles arrived later in fragments of 1 m^2 than in control plots (Kareiva 1984, 1987). In this experiment the isolation distance of 1 m between fragments was even smaller than that in our study. Thus, the discontinuity in favourable habitat appears to affect the movement and foraging pattern even in mobile organisms.

Ants are important mutualists of numerous aphid species in the grasslands examined. In our study, ants were more abundant in fragments than in control plots and a greater proportion of the aphid colonies was ant-attended in fragments. Ant-attended colonies in fragments were also more intensively visited by ants than colonies in control plots in one year. The importance of ants for aphids varies among species. Most species in our study are ant-tended but may also live without tending ants. *A. malvae* and some others are rarely or never visited by ants. The better services provided by ants in fragments-such as the removal of honeydew and protection from predators (Sudd 1987)-may contribute to the success of ant-tended aphid species. Furthermore, extrafloral nectaries of *Euphorbia cyparissias* L. were more frequently visited by ants in fragments than in control plots (B. Braschler, unpubl.). This may indicate a greater demand for sugar resources by ants in fragments or, alternatively, be a side effect of the increased ant density. The increased demand for sugar should lead to a more intensive tending of the aphids (Offenberg 2000, 2001). However, non-attended aphid colonies were also more numerous in fragments. This suggests that other factors than enhanced ant services contributed to the increased aphid density found in fragments.

Our study shows that small-scale fragmentation can lead to an increased density of herbivores. It is noteworthy that this effect was found in slightly different aphid communities which consisted predominantly of specialists for single host plant species. Furthermore,

the experimental fragmentation affected the mutualistic aphid-ant interaction. Not all aphid species were ant-attended. The effect on the mutualistic aphid-ant interaction may therefore change the competitive interaction between attended and non-attended aphids. Similarly, other studies demonstrated fragmentation effects on species interactions, mostly on parasitism and predation (Kareiva 1984, 1987, Kruess and Tscharntke 1994, 2000, Lei and Hanski 1997).

The observed fragmentation effects on aphid density might be a combined result of several distinct influences including a higher abundance of mutualistic ants, an increased plant productivity and altered abiotic factors in fragments. Other potential influences like reduced predator pressure could not be demonstrated but may also contribute to a higher aphid density in fragments.

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Appendix A: Aphid species and density (individuals/0.25 m^2) recorded in experimental plots in 1998. In a few cases the aphid species could not be determined (only nymphs or alatae were found). Frequency indicates in how many experimental plots (fragments and control plots) a particular species was recorded at the two study sites (N: Nenzlingen, 40 plots; V: Vicques, 32 plots). Aphid densities of single species were calculated on the basis of plots occupied by the particular species (means $\pm$ 1SD). The number of plots is given in parentheses. Ant species observed at aphid colonies: Lf: *Lasius flavus* (Fabricius), Lp: *L. paralienus* Seifert, M: *Myrmica* sp.

Aphid species	Host plants	Frequency		Aphid density		Ant species
		N	V	Fragments	Control plots	
Acyrthosiphon loti (Theobald, 1913)	*Lotus corniculatus*		1	–	0.01 (1)	–
A. malvae poterii Prior et Stroyan, 1964	*Sanguisorba minor*	13	6	1.50 ± 2.37 (13)	0.12 ± 0.10 (6)	–
Aphis acetosae Linnaeus, 1761	*Rumex acetosa*	1		0.30 (1)	–	Lp
A. confusa Walker, 1849	*Knautia arvensis*, *Scabiosa columbaria*	6		11.25 ± 9.93 (4)	13.79 ± 11.93 (2)	Lp
A. craccivora Koch, 1854	*Lotus corniculatus*	1	3	16.50 ± 29.12 (4)	–	Lp, M
A. euphorbiae Kaltenbach, 1843	*Euphorbia cyparissias*	1		3.64 (1)	–	Lp
A. helianthemi Ferrari, 1872	*Helianthemum nummularium*		2	7.96 ± 5.71 (2)	–	Lp, M
A. plantaginis Goeze, 1778	*Plantago lanceolata*	2		1.05 (1)	0.21 (1)	Lp
	Plantago media		1	0.89 (1)	–	Lp
A. pomi DeGeer, 1773	*Crataegus monogyna*		1	0.14 (1)	–	Lp
A. sanguisorbae Schrank, 1801	*Sanguisorba minor*	2		0.03 ± 0.02 (2)	–	Lp
Aphis sp.	*Salvia pratensis*	4		4.46 ± 7.12 (3)	0.07 (1)	Lp
Aphis sp.	*Senecio erucifolius*	1		–	0.25 (1)	–
Aphis sp.	*Origanum vulgare*		1	504 (1)	–	Lp
A. stachydis Mordvilko, 1929	*Betonica officinalis*	9	6	1.37 ± 1.67 (9)	12.78 ± 29.03 (6)	Lf, Lp
Brachycaudus sp. (alatae and nymphs)	unknown		1	0.05 (1)	–	Lp
Dysaphis (*Pomaphis*) *plantaginea* (Passerini, 1860)	*Plantago media*	1	5	0.07 ± 0.10 (3)	0.02 ± 0.01 (3)	–
Macrosiphum rosae (Linnaeus, 1758)	*Knautia arvensis*	6		22.15 ± 31.17 (5)	25.00 (1)	Lp
	Rosa sp.		1	–	0.10 (1)	–
Myzaphis rosarum (Kaltenbach, 1843)	*Rosa* sp.		1	0.01 (1)	–	–
Myzus (*Galiobium*) *langei* (Börner, 1933)	*Galium verum*		1	44.44 (1)	–	Lp
Myzus sp. (alatae)	unknown		1	–	0.01 (1)	–
Semiaphis sp.	unknown		1	–	0.06 (1)	Lp
unidentified species (escaped)	*Veronica* sp.	1		–	0.01 (1)	–
unidentified species (nymph)	*Hieracium pilosella*		1	–	0.01 (1)	–
unidentified species (alatae)	unknown		1	–	0.01 (1)	–

Appendix B. Plant taxa infested by aphids in Nenzlingen (N) and Vicques (V) in the years 1997–1999.

Plant species	1997		1998		1999	
Agrimonia eupatoria L.	N				N	V
Anthyllis vulneraria L.					N	
Betonica officinalis L.	N	V	N	V	N	V
Centaurea jacea L.		V				V
Chamaespartium sagittale P. Gibbs					N	
Crataegus monogyna Jacq.	N			V		
Daucus carota L.					N	V
Euphorbia cyparissias L.			N		N	
Galium verum L.	N			V	N	V
Genista tinctoria L.						V
Helianthemum nummularium Miller				V	N	
Hieracium pilosella L.				V	N	V
Hypericum perforatum L.	N	V			N	V
Knautia arvensis Duby	N	V	N		N	V
Lathyrus pratensis L.	N				N	
Lotus corniculatus L.	N	V	N	V	N	V
Ononis repens L.	N				N	
Origanum vulgare L.	N			V	N	V
Plantago lanceolata L.		V	N			
Plantago media L.	N		N	V	N	V
Prunella grandiflora Scholler		V			N	V
Prunus spinosa L.					N	V
Rosa sp.				V		
Rumex acetosa L.			N			
Salvia pratensis L.	N	V	N	V	N	
Sanguisorba minor Scop.	N		N	V	N	V
Scabiosa columbaria L.			N		N	
Senecio erucifolius L.			N			V
Thymus serpyllum L.					N	
Trifolium sp.					N	V
Veronica sp.	N		N		N	
Viola hirta L.					N	