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Plant population size and isolation affect herbivory of *Silene latifolia* by the specialist herbivore *Hadena bicruris* and parasitism of the herbivore by parasitoids

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Abstract Habitat fragmentation can affect levels of herbivory in plant populations if plants and herbivores are differentially affected by fragmentation. Moreover, if herbivores are top-down controlled by predators or parasitoids, herbivory may also be affected by differential effects of fragmentation on herbivores and their natural enemies. We used natural *Silene latifolia* populations to examine the effects of plant population size and isolation on the level of herbivory by the seed pre-dating noctuid *Hadena bicruris* and the rate of parasitism of the herbivore by its parasitoids. In addition, we examined oviposition rate, herbivory and parasitism in differently sized experimental populations. In natural populations, the level of herbivory increased and the rate of parasitism decreased with decreasing plant population size and increasing degree of isolation. The number of parasitoid species also declined with decreasing plant population size. In the experimental populations, the level of herbivory was also higher in smaller populations, in accordance with higher oviposition rates, but was not accompanied by lower rates of parasitism. Similarly, oviposition rate and herbivory, but not parasitism rate, increased near the edges of populations. These results suggests that in this system with the well dispersing herbivore *H. bicruris*, habitat fragmentation increases herbivory of the plant through a behavioural response of the moth that leads to higher oviposition rates in fragmented populations with a reduced population size, increased isolation and higher edge-to-interior ratio. Although the rate of parasitism and the

number of parasitoid species declined with decreasing population size in the natural populations, we argue that in this system it is unlikely that this decline made a major contribution to increased herbivory.

Keywords Oviposition rate · Parasitoids · Isolation · Population size · Edge effects

Introduction

Major consequences of natural and human caused habitat fragmentation are a reduction of habitat area and population size, an increase in isolation, and an increase of the edge-to-interior ratio of habitats and populations (Saunders et al. 1991). Small populations are vulnerable to environmental (Lande 1993; Menges 1998), demographic (Kery et al. 2003) and genetic stochasticity (Ellstrand and Elam 1993). Furthermore, the increase in isolation can greatly reduce the exchange of genetic material through pollen and dispersing individuals and diaspores (Richards et al. 1999), potentially causing inbreeding depression that reduces the fitness of individuals and decreases the viability of the population (Frankel and Soulé 1981; Richards 2000). Due to these processes, small and fragmented populations can become more prone to extinction (Schemske et al. 1994; Fischer and Stöcklin 1997; Courchamp et al. 1999; Matthies et al. 2004).

However, not only these direct effects of fragmentation, but also indirect effects through changes in biotic interactions may affect population viability and the performance of species in small and isolated fragments (Kareiva 1987; Ouborg and Biere 2003). Examples are the effects of fragmentation on the interactions between plants and their pollinators (e.g. Aizen and Feinsinger 1994; Ågren 1996; Steffan-Dewenter and Tscharntke 1999; Groom 2001), their herbivores (Zabel and Tscharntke 1998; Ehlers and

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Olesen 2003) and their pathogens (Groppe et al. 2001) and the resulting effects on plant fitness.

Habitat fragmentation often leads to a decrease in the number of pollinator species present (e.g. Steffan-Dewenter and Tscharrntke 1999), a reduction in the number of successful pollinations, leading to a smaller seed set (e.g. Jennersten 1988; Ågren 1996; Schulke and Waser 2001), and potentially to a lower offspring number in small and isolated plant populations (Groom 2001). On the other hand, a commonly observed result of fragmentation is a reduced number of pathogen or herbivore species present in small or isolated habitat populations (Zabel and Tscharrntke 1998; Kruess and Tscharrntke 2000; Colling and Matthies 2004). This can reduce the level of plant damage (Groom 2001) and shows a potential positive effect of habitat fragmentation on plant performance (Colling and Matthies 2004), especially by a reduction of specialist herbivore species (Zabel and Tscharrntke 1998).

The number of herbivore individuals can strongly depend on the presence of parasitoids and predators (Hairston et al. 1960; Hochberg and Ives 2000). Several studies suggest that this third trophic level is more strongly affected by habitat fragmentation than the second trophic level (Kareiva 1987; Tscharrntke and Brandl 2004), leading to a reduction in parasitism in isolated and small habitats (Kruess and Tscharrntke 1994; Roland and Taylor 1997). This might cause an increase in local herbivore populations (Roland 1993) and might have an immediate effect by increasing the amount of plant tissue damaged by insect larvae (Guillot and Vinson 1973; Powell 1989; Elzinga et al. 2003).

Not only the presence of animal species, but also their behaviour may change in response to fragmentation. Several studies have shown that dispersal behaviour can be altered by population size, resulting in an increase in the number of individuals dispersing from a small population (Sheehan and Shelton 1989; Bergman and Landin 2001). Also, a larger proportion of individuals may stay or return in fragmented populations (Baguette et al. 2003). Differences in migration rate will obviously affect the number of individuals in a population and therefore the level of herbivory or parasitism rate. Also, oviposition rate of insects can be affected by the size of plant populations (Maguire 1983; Shea et al. 2000), leading to differences in the level of herbivory.

Whereas the decrease in population size and the increase in isolation are recognised as important factors leading to changes in the level of herbivory, the effects of changes in edge-to-interior ratio are less well studied (Fagan et al. 1999). Biotic interactions near edges may be different from those in the centre of a population, leading to changes in the level of pollination (Jules and Rathcke 1999), herbivory (Lienert and Fischer 2003) or parasitism rate (Roland 1993; Cronin 2003b). As small populations usually have larger edge-to-interior ratios, these populations can be more strongly affected.

We studied effects of habitat fragmentation of the plant *Silene latifolia*, on the level of herbivory by the specialist noctuid herbivore *Hadena bicruris* and on the rate of parasitism of the herbivore by its parasitoids. *S. latifolia* suffers high levels of pre-dispersal seed predation by *H. bicruris* (Biere and Honders 1996b; Wolfe 2002). On average 50% of the herbivores is attacked by generalist and specialist parasitoids (Elzinga 2005). In natural populations, we tested effects of plant population size and isolation on herbivory and parasitism. In addition, we used large and small experimental populations to test the effects of population size and distance to the edge of the population on oviposition, herbivory and parasitism. We hypothesised that the herbivore, a good disperser, would not be more frequently absent from small, isolated populations, but that the number of parasitoid species and levels of parasitism would be lower in such populations, resulting in increased levels of herbivory.

Methods

Study system

Silene latifolia Poirlet (= *S. alba* (Miller) Krause = *Melandrium album* (Miller) Garcke), the white campion, is a dioecious, weedy, short-lived perennial occurring in open disturbed habitats like fallow fields, field margins and roadsides (Goulson and Jerrim 1997). It flowers from the end of April up to the end of October, but usually has two distinct flowering peaks at the end of May and the beginning of August (Biere and Honders 1996a; Bopp 2003). Pollination in Europe is mainly performed by moths, with *H. bicruris* Hufn. (Lepidoptera: Noctuidae), the Lychnis, and *Autographa gamma* as most common visitors (van Putten 2002). After pollination of the female flower, the ovule expands and develops into a seed capsule containing several hundreds of seeds (Jürgens et al. 1996). In Western Europe, the specialist *H. bicruris*, a night-active moth with a wingspan of 30–40 mm, is the most important herbivore consuming the developing seeds of *S. latifolia* (Brantjes 1976b; Wirooks and Plassmann 1999; Wolfe 2002). In the Netherlands, its caterpillars can be found from May until October (personal observation), but usually two peaks in June and at the end of August occur, indicating that there are at least two overlapping generations. After pollination the female moth may oviposit a single egg on the ovary of female flowers of *S. latifolia* (Brantjes 1976b). Directly after hatching the first instar caterpillar chews a small hole in the side of the ovary, enters the young fruit, and starts to feed on the ovules and developing seeds. It will stay in this seed capsule (hereafter called primary capsule) until it has consumed all the seeds, which usually occurs when the caterpillar has reached its fourth or fifth host instar. At this stage, the caterpillar leaves the primary capsule through the enlarged

entrance hole and starts feeding on other seed capsules from the top (hereafter called secondary capsules). Late instar caterpillars are usually too large to be self-contained within a seed capsule, and thus the caudal appendages usually protrude from the capsule during feeding bouts, exposing it to parasitoids (Biere et al. 2002). It is this unprotected stage of *H. bicruris* that is most vulnerable to its parasitoids.

Natural populations

We studied the level of herbivory and the level of parasitism of the herbivores by parasitoids in natural *S. latifolia* populations along 100 km of the rivers Rhine and Waal, the main branch after splitting of the Rhine, in the Netherlands from the German border to approximately the city of Gorinchem. In this area, we have located all natural populations of *S. latifolia*. The area consists of floodplains and river dikes with different types of management, soil and elevation. *S. latifolia* populations mainly grow on the elevated sandier parts and river dikes, but also in disturbed areas near industrial sites and along roadsides and field margins. The surrounding area was unsuitable for *S. latifolia* due to heavy agriculture and less suitable soil. Due to these clear borders, we are confident that we located all populations present in the study area.

We visited all populations several times a year in 2001, 2002 and 2003. We determined the size of all populations by counting the number of reproducing plants (plants with flowers, seed capsules, or flower buds) in August 2001, early June 2002 and early June 2003. Furthermore, for each population, we computed distance to the nearest population from coordinates of plants at the borders of the populations measured with a GPS system (GIS *backpack*, Commetius, Leiderdorp, NL). Populations were defined as groups of *S. latifolia* plants separated from conspecifics by at least 100 m in all directions.

Every year, a few weeks after a flowering peak when most plants had ripe seed capsules, we assessed the level of herbivory in all populations (in 2001 in September, in 2002 in July, in 2003 in July) that had not been mown or otherwise disturbed. We randomly selected a maximum of approximately 100 female plants per population, and counted how many of these had at least one seed capsule that showed a sign of herbivory. Second, from at least ten randomly selected female plants, or from all plants if ten or less were present, we counted all undamaged seed capsules and capsules showing signs of herbivory. In most capsules with herbivore damage all seeds were completely eaten. Capsules attacked by *H. bicruris* can be easily recognised either by the presence of a hole in the side of the capsule (a primary seed capsule) or by the presence of a large round hole on the top (a secondary seed capsule).

At every visit, we collected caterpillars that were observed either on the plant or inside a secondary seed

capsule. Collected caterpillars were reared through in the lab on artificial diet (Elzinga et al. 2002) at 25/15°C 16/8 h L/D to find out whether they were parasitized. From September onwards caterpillars were reared at 8/16 h L/D to mimic autumn conditions and initiate diapause in both caterpillars and parasitoids. Diapausing insects were placed 6 months at 4°C 16/8 L/D prior to continued rearing. Caterpillars that died were dissected to determine whether they had been parasitised.

Experimental populations

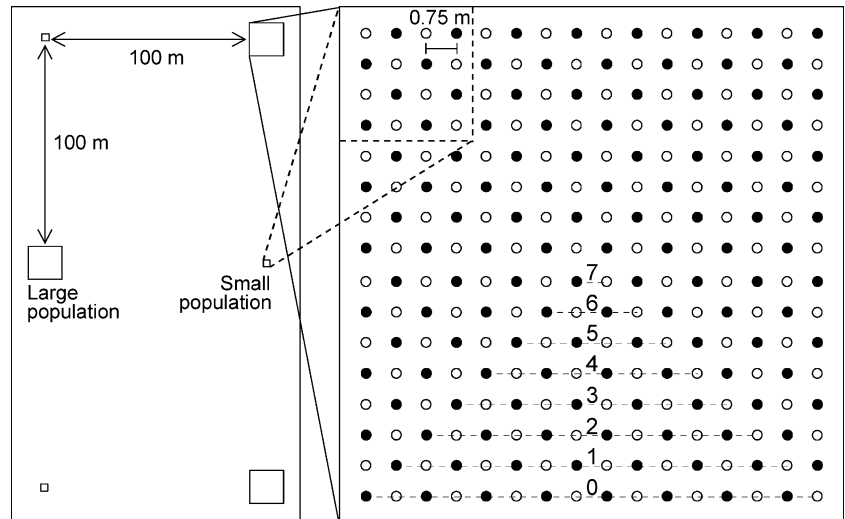
To study the effects of population size on herbivory and parasitism rate, we created experimental plant populations of *S. latifolia* in a meadow field at the Netherlands Institute of Ecology in Heteren, the Netherlands, at approximately 20 km from the natural study area. An experimental garden, located a few hundred meters away, where *S. latifolia* had been grown for several years served as a source for both the herbivore and its main parasitoids that had colonised the experimental garden over the years from natural populations located a few kilometres away.

We created three small and three large populations separated 100 m from each other (the distance used to distinguish populations in the natural study area) in 2002 and in 2003 (Fig. 1). Small populations consisted of 16 plants arranged in a square (4×4) with 0.75 m in between plants. Male and female plants were placed alternating in 2002 and randomly in 2003. The large populations were similarly arranged but consisted of 256 plants (16×16 grid) creating groups of plants at eight different distances from the edge (Fig. 1). In each year, plants were grown from seedlings in large pots (18×18×18 cm) with a mixture of clay, peat and sand (respectively 12, 68, and 20% vol). They were watered when necessary.

In 2002, we made weekly counts of the number of eggs deposited on female flowers. Eggs are usually deposited during pollination after which flowers start to wilt. Wilted flowers were counted and removed weekly. All the flowers were carefully checked for the presence of eggs or small larvae of *H. bicruris*. Due to the large number of flowers, counting and checking of flowers from large populations was restricted to one of four quadrants (every week a different quadrant). In the remaining parts of the large populations, we removed all wilted flowers but did not count or check them for eggs.

In 2003, we measured both the level of herbivory and the level of parasitism. Every week we checked all the plants for caterpillars. We collected all caterpillars that were either on the plant or inside a secondary seed capsule and reared them through as described above. Furthermore, every week the damaged seed capsules and those that appeared ripe were removed and carefully checked for signs of primary or secondary herbivory by *H. bicruris*. Parasitoids emerging from parasitised caterpillars were released as adults at the location where its

Fig. 1 Setup of small (16 plants) and large (256 plants) experimental populations of *S. latifolia* in 2002. *Open symbols* males, *closed symbols* females. In 2003, male and female plants were distributed randomly and large and small populations were transposed. Distances to the edge (in numbers of rows) are indicated for one side of the large population



host had been collected to allow species to persist in the populations during the year.

Analyses

For natural populations, the level of herbivory was calculated both at the population level (as the proportion of counted plants that had at least one capsule damaged) and at the plant level (as the proportion of the counted seed capsules that were damaged). Oviposition, herbivory and parasitism rates were analysed using type III generalised linear models with a binomial error distribution and a logit link function (SAS v. 8.2, procedure GENMOD, SAS Institute, Cary, NC, USA). Quasi-*F* values were calculated as the ratio of effect mean deviances and the appropriate error mean deviances. A scale parameter was estimated to correct for over- or underdispersion (McCullagh and Nelder 1989). We analysed effects of plant population size and distance to the nearest neighbouring population (both log-transformed) on herbivory in natural populations for each of the 3 years separately. For analyses of parasitism rate in natural populations data from the 3 years were pooled per population, since especially in many of the small populations only few or no caterpillars could be collected in a single year. Similar analyses as for oviposition rate were used to test effects of plant population size (the maximum number of plants) and distance to the nearest neighbouring population on the number of parasitoid species found in a population and on the proportion of parasitised caterpillars in a population.

For the experimental populations, we analysed the effects of population size (large vs. small populations), replicate population (nested within population size) and time (weekly counts) on the proportion of flowers with at least one egg (data 2002). As models based on analyses of oviposition as a binomial variable (eggs present/absent) did not converge, these analyses were based on

scores of the proportion of oviposited flowers per plant, which were approximately normally distributed and analysed by generalised linear models with a normal distribution and an identity link function. The level of herbivory and the parasitism rate in experimental populations (data 2003) were analysed similarly, but here we could only use the total numbers over the whole experimental period. In addition, for large populations, we analysed effects of the position of plants relative to the edge of the population, time and replicate population on oviposition rate, level of herbivory and parasitism rate.

Results

Natural populations

In total, we located 85 populations of *S. latifolia* in the study area ranging in size from 1 to 13,000 plants (median=21 plants). About 50 populations (median=39 plants) each year were suitable to estimate the level of herbivory, the rest was disturbed by mowing, or contained only male plants. *H. bicruris* was present in almost every population we visited each year (Fig. 2). In general, the level of herbivory was very high. On average 80% of all plants and 50% of all seed capsules that were investigated showed signs of herbivory by *H. bicruris*.

The proportion of plants damaged by *H. bicruris* (with at least one seed capsule showing signs of herbivory) decreased with the number of plants in a population in 2001 and 2002 (Fig. 2a, b, Table 1), but was not affected by distance to the nearest population in any of the years (Table 1). The proportion of seed capsules infested by *H. bicruris* decreased with increasing population size in 2002 and 2003 (Fig. 3c, e, Table 1) and increased with distance to the nearest population in 2001 and 2002 (Fig. 3b, d, Table 1). The decrease with population size in 2002 was stronger in isolated than in connected populations (Fig. 3c, Table 1).

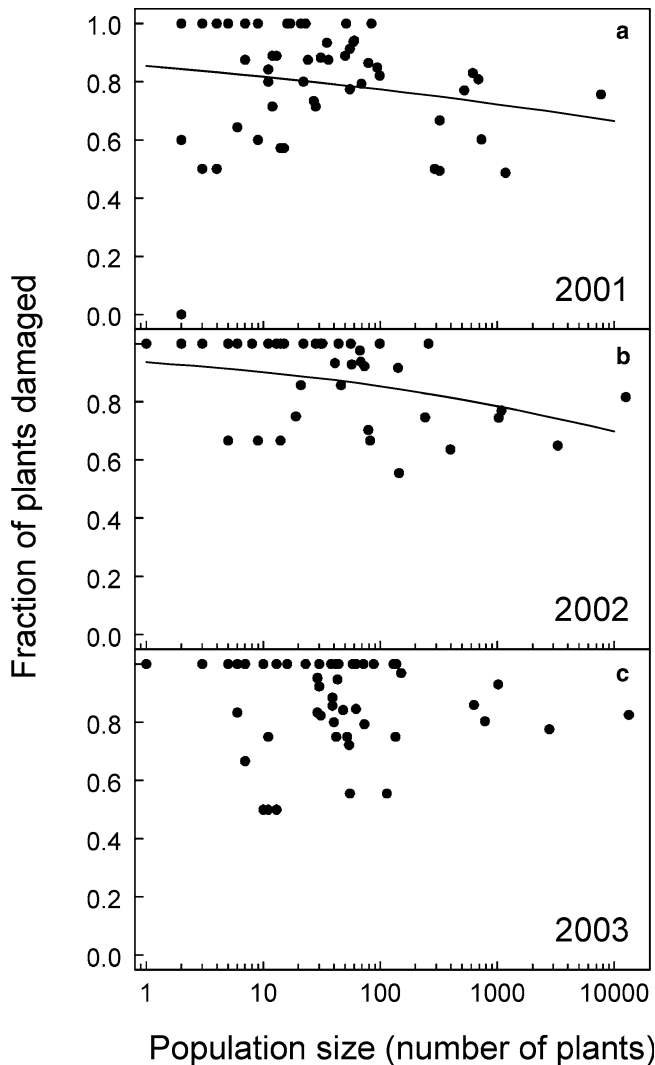


Fig. 2 a–c Proportion of *S. latifolia* plants damaged by *H. bicruris* (at least one seed capsule damaged) in natural populations as a function of plant population size in **a** 2001, **b** 2002 and **c** 2003. Fitted lines are based on parameter estimates from analyses presented in Table 1

In about 80% of all populations, we found at least one caterpillar that was parasitised. In total, approximately 50% of the caterpillars were parasitised. At least 14 parasitoid species were responsible for these parasitisms, but their contribution was unequal; only four hymenopteran species accounted for 96.7% of the parasitisms. These were the small gregarious koinobiont *Microplitis tristis* (Braconidae) (51.9%), the large solitary larval–pupal parasitoid *Eurylabus tristis* (Ichneumonidae) (26.4%), the small gregarious ectoparasitoid *Bracon variator* (Braconidae) (10.6%) and the large solitary koinobiont *Ophion pteridis* (Ichneumonidae) (7.8%). The total number of parasitoid species observed in a population during the 3 years of study increased with the maximum number of host plants observed in the population during this period (Table 1) but was unaffected by the degree of isolation

of the population. The parasitism rate of caterpillars also increased with the maximum population size of host plants (Fig. 4, Table 1) and in addition decreased with the distance to the nearest population (Fig. 4, Table 1). The increase in parasitism rate with population size was stronger for less isolated than for isolated populations (Fig. 4, Table 1, interaction). To assess the effect of the number of parasitoid species on parasitism rate, we repeated the analysis of Table 1d with the number of parasitoid species included as an independent variable. There was no effect of the number of parasitoid species on parasitism rate ($F_{1,55}=0.8$, $P=0.39$), whereas effects of population size and isolation and their interaction were all significant.

Experimental populations

In 2002, we were able to check 6,854 female flowers for the presence of *H. bicruris* eggs during 12 weeks from May 24 until August 9, after which flower production by the plants rapidly ceased. More than 95% of the plants flowered during the experiment and in more than a quarter of all the flowers the herbivore had deposited one or more eggs. The oviposition rate was higher in small (35.9%) than in large (25.4%) populations ($F_{1,4}=35.6$, $P<0.01$, Fig. 5a). Oviposition rate also differed among replicate populations within the two size classes ($F_{4,55}=20.9$, $P<0.001$) and varied with time ($F_{11,55}=17.5$, $P<0.001$). Interactions between these factors were not significant. Although most eggs were laid singly (one per flower), 31.5 and 17.3% of the eggs were found in flowers that contained more than one egg (2–5) in small and large populations, respectively (Fig. 5b); the difference was not statistically significant ($F_{1,4}=3.61$, $P=0.13$). In 2003, we checked 6,444 seed capsules for primary or secondary predation. The proportion of seed capsules that were predated was higher in small (50.4%) than in large (33.9%) populations (Fig. 5c; $F_{1,4}=17.5$, $P<0.05$), mainly due to a higher proportion of capsules that were consumed by large *H. bicruris* caterpillars that had left their primary seed capsule (secondary predation, Fig. 5c). Overall parasitism rate of caterpillars was high (Fig. 5d), but it did not differ between differently sized populations ($F_{1,4}=4.0$, $P=0.11$, Fig. 5d).

In the three large populations, oviposition rate overall decreased with the distance of a plant to the edge of the population although the strength of the edge effect varied among populations (Table 2, Fig. 6). The proportion of damaged seed capsules on a plant also decreased towards the centre of the populations (Table 2, Fig. 6). For the level of parasitism, edge effects were inconsistent among populations. In one of the populations, parasitism decreased towards the centre, in the second, it increased and in the third, no effect was observed (Table 2, Fig. 6).

Table 1 Effects of population size and isolation (distance to nearest population)

Source	df	Percentage of damaged plants ^a			Percentage of damaged capsules ^b			No. of parasitoid species ^c	Parasitism ^d (%)
		2001	2002	2003	2001	2002	2003		
Population size	1	5.0*	16.0***	2.0	3.6	21.0***	4.7*	37.4***	31.8***
Isolation	1	0.2	2.7	0.0	7.9**	3.7	3.1	0.5	6.3*
Size × isolation	1	–	–	–	–	4.8*	–	–	8.6**
Error df		47	41	50	47	40	50	62	56

Values are pseudo-*F* values from GLM. Non-significant interaction terms (–) are pooled with the error term. Data for natural populations in three different years, except for (c) and (d) (data from different years pooled into one estimate per population)

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

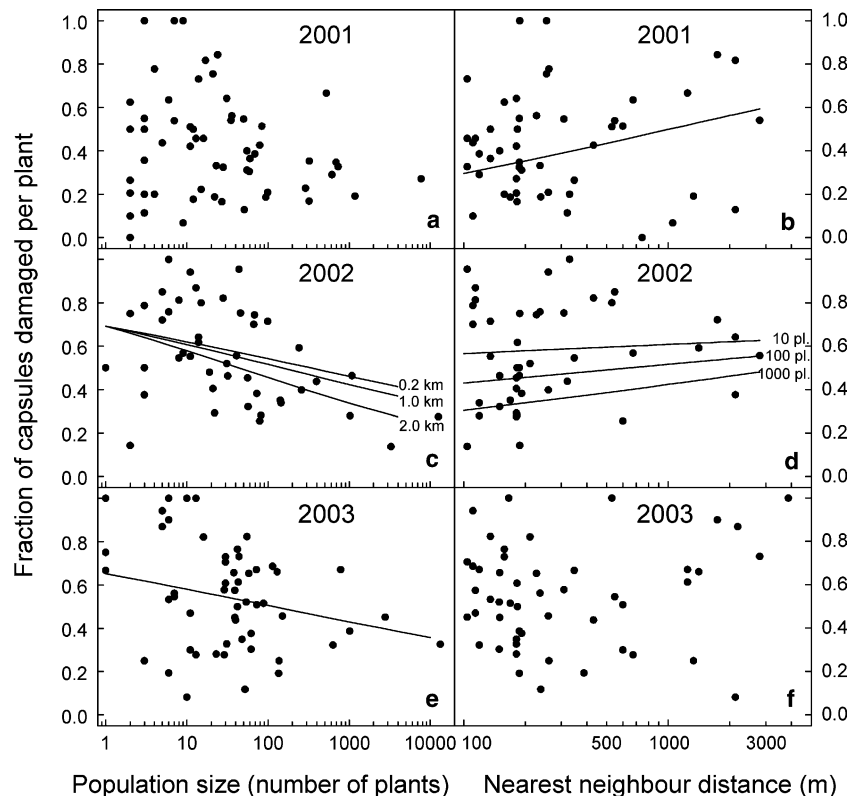
^aThe proportion of *S. latifolia* plants damaged by *H. bicurris* (at least one seed capsule damaged)

^bThe proportion of seed capsules damaged per plant

^cThe number of parasitoid species observed in a population

^dThe proportion of *H. bicurris* parasitised by parasitoids

Fig. 3 a–f Proportion of seed capsules of *S. latifolia* damaged by *H. bicurris* as a function of plant population size and isolation of natural plant populations in: **a, b** 2001, **c, d** 2002 and **e, f** 2003. Fitted lines are based on parameter estimates from analyses presented in Table 1. Interaction effects are represented by *lines* for three different values of distance to the nearest population (*nm*) and population size (*pl* number of plants)



Discussion

Effect of population size and isolation on herbivory and parasitism in natural populations

Hadena bicurris was present in almost every natural *S. latifolia* population in each of the years. This result is consistent with earlier studies (Elzinga 2005) that *H. bicurris* easily colonises small experimental plant populations located more than 2 km away from the nearest source population within a single generation. In each of the 3 years of study, we found that the level of herbivory was higher in smaller and/or more isolated

populations. Several studies in natural systems have shown effects of habitat fragmentation on herbivory, either in a similar direction as we observed (Braschler et al. 2003; Lienert and Fischer 2003) or in the opposite direction, a decrease in herbivory in small or isolated populations. The latter is often attributed to corresponding differences in plant density, plant characteristics or Allee effects (Groom 2001; Kery et al. 2001; Ehlers and Olesen 2003; Colling and Matthies 2004). We suggest that observations of decreased herbivory in small isolated populations may be more likely in systems with less well dispersing herbivores than in our study system.

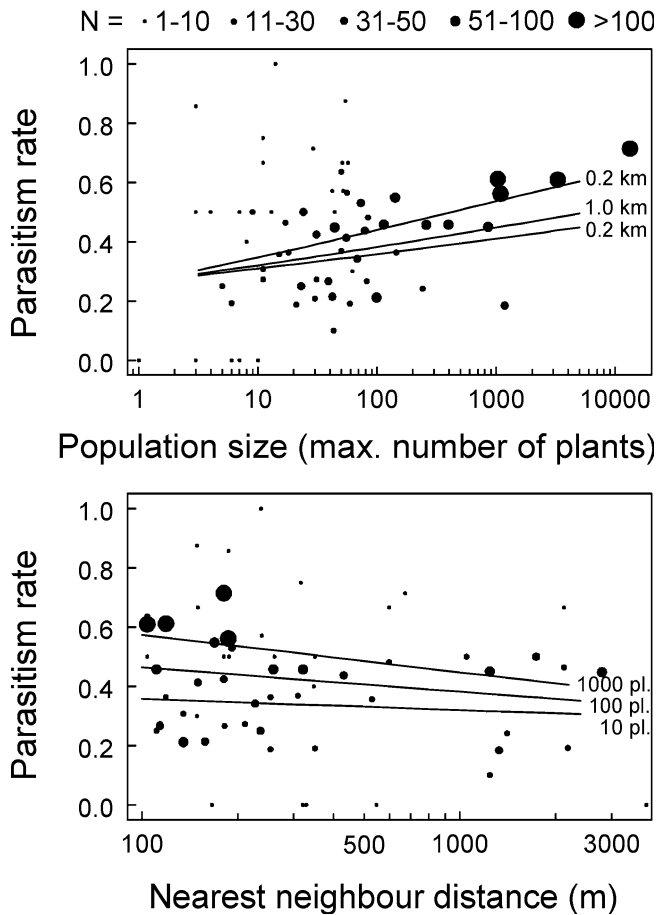


Fig. 4 Fraction of *H. bicurris* caterpillars parasitised by parasitoids in natural *S. latifolia* populations over the 3 years of study as a function of plant population size (*top panel*) and isolation (*lower panel*). The size of a dot indicates the sample size of caterpillars (hosts) on which the datapoint is based. *Fitted lines* are based on parameter estimates from analyses presented in Table 1. Interaction effects are represented by *lines* for different values of distance to the nearest population (*md*) or population size (*pl* number of plants)

The higher level of herbivory in small, isolated populations could be partly due to a reduced rate of parasitism of the herbivore, as suggested in a number of studies (Roland 1993; Kruess and Tscharrntke 1994). In our system, the higher level of herbivory in smaller populations was indeed accompanied by a lower number of parasitoid species and a lower rate of parasitism. Other studies have documented similar decreases in parasitoid species number and rate of parasitism with decreasing population size (Segarra-Carmona and Barbosa 1992; Kruess and Tscharrntke 2000). However, we argue that it is unlikely that the lower number of parasitoid species or the lower rates of parasitism made a significant contribution to the higher levels of herbivory in the smaller natural populations.

First, although the number of parasitoid species in a population increased with plant population size, we could not detect an effect of the number of parasitoid species on the rate of parasitism in a population. Effects

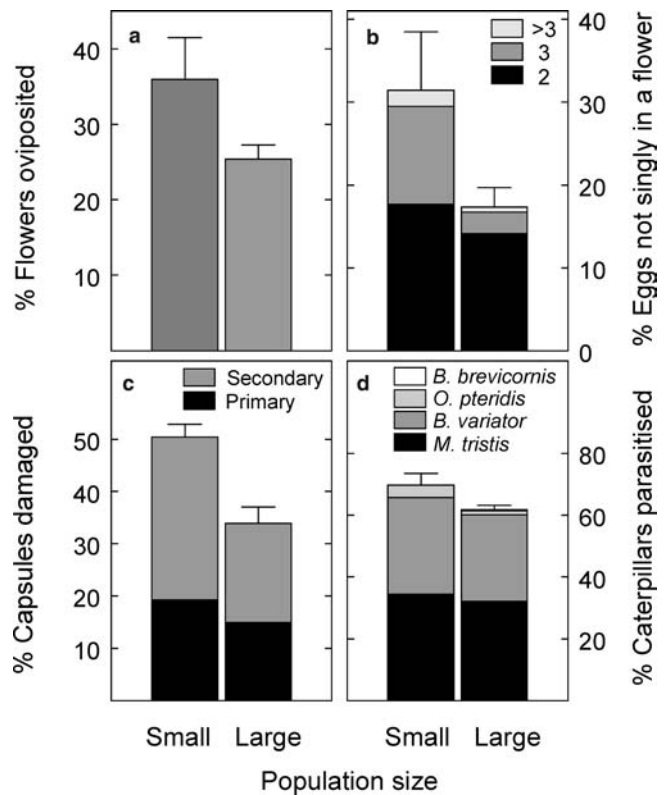


Fig. 5 a-d Differences between small and large experimental populations of *S. latifolia* in: **a** the proportion of flowers with at least one egg of *H. bicurris*, **b** the proportion of eggs found in flowers that contain multiple (2–5) eggs, **c** the proportion of seed capsules damaged by *H. bicurris*, divided into primary and secondary herbivory (see text) and **d** the proportion of caterpillars parasitised by the indicated parasitoid species. *Bars* indicate 1 SE of the mean

of the number of parasitoid species on total parasitism rate have rarely been studied, but probably depend strongly on the identity of the species involved (Doak 2000). In our study, only four parasitoid species accounted for more than 95% of the parasitisms, whereas the ten other species parasitising *H. bicurris* in the study area caused only a very small fraction of the parasitisms (Elzinga 2005). Only one out of these four main parasitoid species was affected by isolation (Elzinga 2005), which may explain why we did not find an overall significant effect of isolation on the number of parasitoid species. This is in contrast with other studies, which showed a strong decrease in parasitoid species number and parasitism rate with increasing isolation (Kruess and Tscharrntke 1994).

Second, the effects of parasitism on herbivory through direct reduction of the quantity of food eaten by individual caterpillars may be small in our system. All of the four major parasitoids attack caterpillars in their final stages, when a considerable amount of damage by the caterpillar has already been done. Only *B. variator*, which was found on ca. 5% of the caterpillars, arrests host growth and feeding; the others

Table 2 Effects of plant position (distance from the edge of the population), time (week) and replicate population

	df	Oviposition ^a 2002	Damage ^b 2003	Parasitism ^c 2003
Distance to edge (<i>D</i>)	1	11.5***	6.8**	1.4
Time (<i>T</i>)	11	20.7***	ND	ND
Replicate (<i>R</i>)	2	5.4**	7.9***	3.3*
<i>D</i> × <i>R</i>	2	3.4*	–	3.3*
<i>T</i> × <i>R</i>	22	3.4***	ND	ND
Error df		890	365	226

Data for three large experimental populations of 256 plants in 2002 and 2003. Values are pseudo-*F* values from GLM. Non-significant interaction terms (–) are pooled with the error term
 ND not determined

P* < 0.05; *P* < 0.01; ****P* < 0.001

^aThe proportion of flowers per *S. latifolia* plant with an *H. bicruris* egg

^bThe proportion of seed capsules per plant damaged by *H. bicruris*

^cThe proportion of caterpillars parasitised by parasitoids

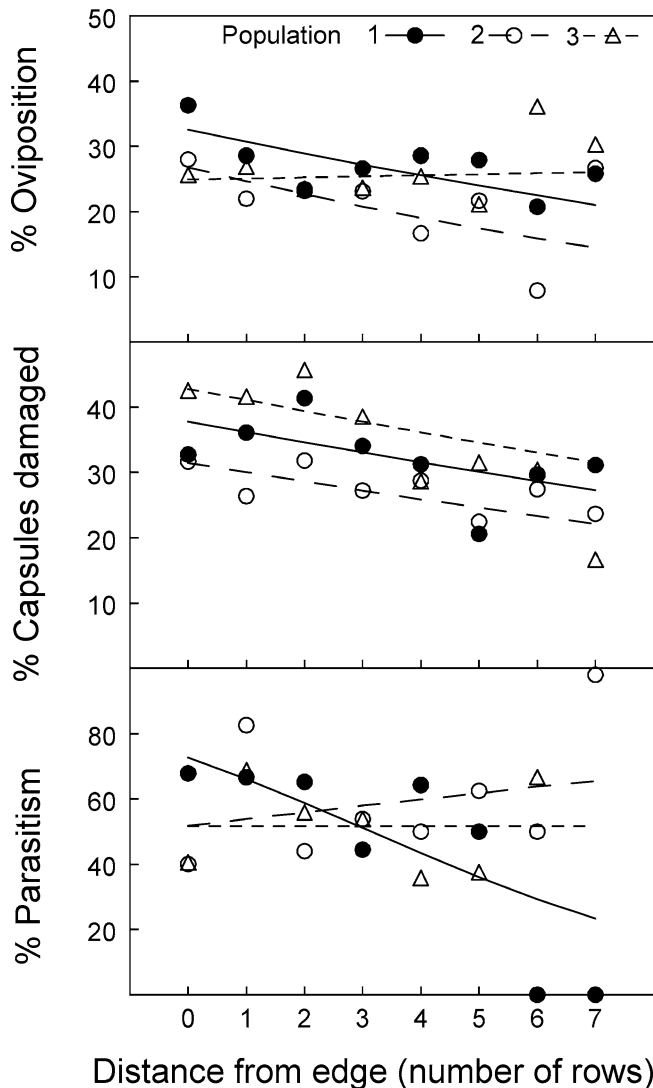


Fig. 6 Effects of distance to the edge of large experimental *S. latifolia* populations (row 0 edge; row 7 centre) on the proportion of flowers with at least one egg of *H. bicruris* (top panel), the proportion of seed capsules damaged by *H. bicruris* (centre) and the proportion of *H. bicruris* caterpillars parasitised (lower panel) in three replicate populations (pop. 1–3). Fitted lines are based on parameter estimates from analyses presented in Table 2

are koinobionts allowing the caterpillar to continue to feed and grow until just prior of eclosion of the larvae (Elzinga 2005).

Third, the consequences of the indirect effects of parasitism on herbivory through reduction of the local population size of the herbivore in the next generation are not immediately clear. For a large range of herbivore population sizes, oviposition in smaller populations may well be limited by the available number of flowers rather than by the number of herbivores. One female herbivore escaping parasitism may deposit an egg in all flowers present in small populations as she can produce a few hundred eggs in her life and around 40 eggs per night (Brantjes 1976b; Elzinga et al. 2002). Therefore, although the number of parasitoid species and the rate of parasitism are lower in smaller populations, this does not necessarily make a significant contribution to the observed higher levels of herbivory in smaller populations.

Effects of population size on herbivory and parasitism in experimental populations

In line with the observation of lower herbivory in larger natural populations, rates of herbivory also decreased with plant population size in the experimental populations. But in contrast to the natural populations, parasitism of herbivores did not increase with population size in the experimental populations. These results suggest that although parasitoids, especially the ectoparasitoid *B. variator*, may have lowered the overall level of herbivory in the experimental populations, they were not the primary cause of differences in the level of herbivory between large and small experimental populations. A more likely explanation for the higher levels of herbivory in the smaller populations is the observed higher rate of oviposition in these populations. This suggests that the moth shows a behavioural response resulting in higher proportions of flowers and plants with *H. bicruris* eggs in the smaller populations. Several mechanisms may underlie such a behavioural response. First, patch or host plant leaving decisions of adult

moths may be affected by population size. In small populations, the moth may stay relatively longer and visit relatively more flowers before it leaves the population or plant, similar to observations that pollinators stay longer in isolated populations (Schulke and Waser 2001). Second, the acceptance rate of flowers of lower value by the moth might become higher in small or isolated populations, as indicated by higher levels of superparasitism (Brantjes 1976a). Indeed, we found two-fold higher rates of superparasitism in small compared to the large experimental populations. However, due to the large variance among populations this difference was not statistically significant. Both of these mechanisms could be caused by a relatively large number of mature eggs present in a female moth in small or isolated populations. An adult female moth can produce around 40 eggs during a single night (Brantjes 1976b; Elzinga et al. 2002), much more than the number of female flowers that is available in some of the smaller populations.

An additional explanation for the higher rates of herbivory, in the small experimental populations might be their relatively large edge-to-interior ratio. Several studies have documented increased oviposition rates (Cappucino and Root 1992) and increased numbers of herbivores or higher levels of herbivory near the edges of populations (Lienert and Fischer 2003). This could be due to changes in dispersal behaviour at habitat boundaries (Sparks and Parish 1995; Fagan et al. 1999), herbivores hesitating to cross population boundaries (Cantrell and Cosner 1999), more favourable microclimate or vegetation at boundaries (Cappucino and Martin 1997), or adaptations in the searching and oviposition behaviour of moths resulting in a preference for plants occurring at overall low densities (Courtney and Courtney 1982). In accordance with these studies, we observed overall increases in the rates of oviposition and herbivory from the interior towards the edges of our large experimental populations. Interestingly, the rate of parasitism in the large experimental populations was not affected by distance to the edge, suggesting again that the higher rates of herbivory near population edges were not caused by reduced parasitism of herbivores but rather by the higher oviposition rates. The spatially uniform parasitism rates in the large experimental populations are in accordance with studies of McGeoch and Gaston (2000) showing that the prevalence of a leaf miner increased near woodland edges but that there was no change in parasitism rate, but contrasts with other studies in which parasitism rates decreased towards population edges (Tscharrntke et al. 2002; Cronin 2003a). Whether edge effects also contribute to the higher levels of herbivory in the small natural populations of *S. latifolia* is unknown. The small experimental populations all had a relative large edge-to-interior ratio, but the small natural populations had very different edge-to-interior ratios due to their different shapes, from almost circular to linear.

Effects of habitat fragmentation on the tritrophic system

Our study has shown that effects of habitat fragmentation, i.e. a decrease in population size, a higher degree of isolation and an increase in edge effects, are all likely to increase the level of herbivory by the specialist noctuid *H. bicruris* in populations of its host *S. latifolia*. However, whether the increased level of herbivory in small populations will affect plant population dynamics is unknown. In our research period, only very few populations went extinct, mostly due to external human causes. Under favourable environmental conditions, *S. latifolia* can persist for several years in a vegetative stage. Because the presence of *S. latifolia* is related to the openness of the vegetation, the effects of environment, e.g. disturbance or succession on populations may be more important than the effect of a reduction in seed set due to herbivory. Nevertheless, in several small natural plant populations seed set was reduced to zero by herbivory, which obviously may have consequences for the population viability. Our study further suggests that habitat fragmentation does not necessarily affect insect herbivory through the effects it has on parasitoid presence and parasitism rate. Although fragmentation may lead to the absence of some parasitoid species and a decrease in parasitism rate, increases in the level of herbivory may mainly be due to a behavioural response of the adult herbivore, leading to a relative increase in the number of eggs deposited in fragmented plant populations. If small and isolated plant populations are located by the herbivore, damage can be increased due to fragmentation, leading to reduced plant population viability.

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