Effects of habitat fragmentation on a tri-trophic system:

Silene latifolia, *Hadena bicruris* and its parasitoids

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Front cover: female flowers of *Silene latifolia* with egg of *Hadena bicruris*, caterpillar of *H. bicruris,* and the parasitoids (from above): *Microplitis tristis*, *Eurylabus tristis*, *Bracon variator* and *Ophion pteridis.*

Effects of habitat fragmentation on a tri-trophic system:

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De effecten van habitatfragmentatie op een tritrofisch systeem:

Silene latifolia, *Hadena bicruris* en haar parasitoïden

(met een samenvatting in het Nederlands)

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Chapter 1

General introduction

Habitat fragmentation

The habitat of a species has been defined as "an area with the combination of resources and environmental conditions that promotes occupancy by individuals of a given species and allows those individuals to survive and reproduce" (Morrison *et al.* 1992). For an animal species a habitat can often be subdivided into several parts with specific resources present in each part. In herbivorous insects, for example, a large difference can exist between habitat suitable for immature (= larval) development and habitat in which an adult finds mates, feeds and disperses. In many cases the habitat for insect larvae is very narrow, due to specific dietary needs, while the habitat of the adults is much larger because they can use several types of resources from a variety of plants in different areas that are unsuitable for their progeny. For the sake of simplicity, the habitat of an insect species is often defined as the area where the suitable conditions for larval development are present (Anthes *et al.* 2003). In most cases the habitat of herbivorous insects can thus be defined as the places where their food plants grow. The habitat of specialist predators or parasitoids of the herbivore can be defined similarly although the actual presence of the herbivore is, of course, necessary.

Most habitats exhibit varying degrees of heterogeneity so that the habitat is divided into distinct habitat patches (Hanski and Gilpin 1996, Hanski 1999). One can envisage such natural levels of fragmentation at virtually every scale from geographical scale e.g. mountains and islands, to landscape scale e.g. archipelago's and forest patches, to local small scale heterogeneity e.g. vegetation gaps or single plants. Habitat fragmentation from a conservationists point of view is usually determined at the landscape level, i.e. 100 m to several kilometres (e.g. Krauss *et al.* 2003a). Many species are believed to live in metapopulation structures, where large populations are subdivided into local smaller populations which may regularly go extinct (Levins 1970, Ouborg 1993, Husband and Barrett 1996, Antonovics *et al.* 1997). Limited migration between habitat patches creates a balance between local extinction and recolonisation of patches, allowing the metapopulation as a whole to persist (Hanski and Gilpin 1996, Hanski 1999).

However, nowadays humans are changing the landscape in a rigorous way and at an ever growing pace. Not only are habitats destroyed due to agricultural and urban development, road construction, deforestation, etc., leading to immediate local extinction of species, but the remaining habitat patches often become exceedingly fragmented (Saunders *et al.* 1991, Henle *et al.* 2004). Three consequences of fragmentation for the remaining habitat patches are distinguished (Fig. 1.1): (1) The size of patches becomes smaller, (2) the connectivity between patches decreases due to a reduction in the size of surrounding patches and/or increasing distances to them and (3) the edge:interior ratio increases. These changes can disturb the processes of recolonisation, migration and the population dynamics of component species. This may affect local population persistence negatively and in the long run it can make entire metapopulations of a species more prone to extinction.

There is now a growing awareness, both amongst empiricists and conservationists, that habitat fragmentation can affect species persistence (Debinski and Holt 2000, Henle *et al.* 2004). In the Netherlands habitat fragmentation (or its opposite process "ontsnippering") has become an important issue in biological conservation over the last two decades. In 1990, the government presented its plan to create the "Ecologische Hoofd Structuur" (EHS) (Ministerie van LNV 1990). By creating and preserving corridors and stepping stones of natural vegetation, existing core nature areas were connected to allow dispersal of species. Because the EHS mainly focused on dispersal of large mammals, in 2000 the government introduced "groenblauwe dooradering" (Ministerie van LNV 2000). This will create a network of small semi-natural landscape elements, such as ditches and hedges, that allows small species to disperse and persist in the agricultural landscape (Henkens and van Raffe 2002).

Local effects of habitat fragmentation

Natural and anthropogenic habitat fragmentation of a landscape can have several effects on the local population dynamics of a species in the remaining patches which ultimately may affect the entire metapopulation of that species.

First, a decrease in patch size usually leads to a reduction in the local population size of a species. If less individuals are present, the population will be more prone to stochastic effects (Shaffer 1981, Lande 1993). In particular, smaller populations are more susceptible to environmental (Menges 1998), demographic (Kery *et al.* 2003) and genetic stochasticity (Ellstrand and Elam 1993) that may increase the chance of extinction of the

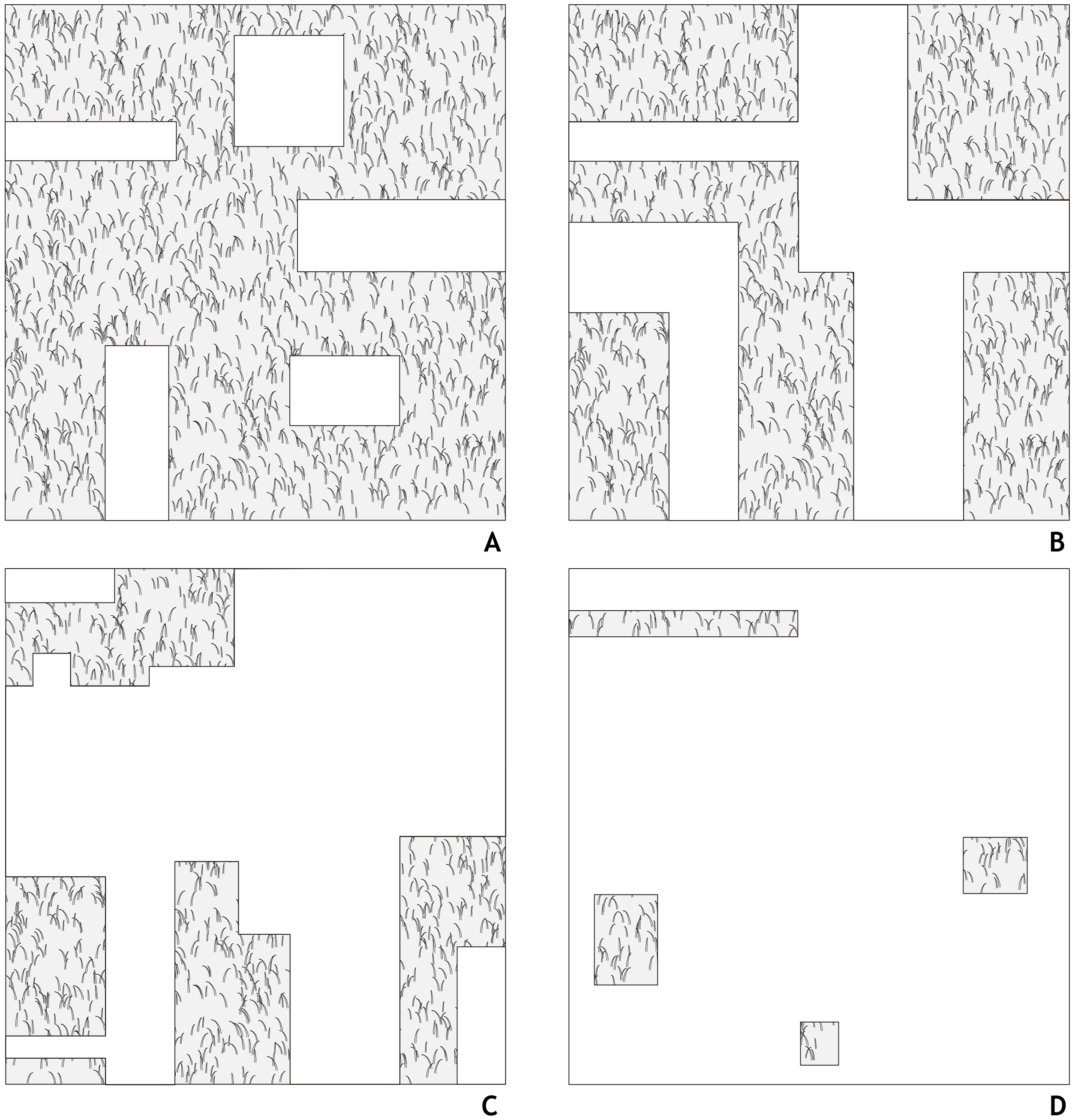


Figure 1.1 Schematic presentation of different levels of habitat fragmentation. (A) A decrease in habitat area. (B) Fragmentation into remnant habitat patches. (C) A decrease in size of the remnant patches and increasing distance between habitat remnants. (D) Only small isolated habitat remnants remain. Note, that from A to D the total habitat area decreases and the edge:interior ratio increases.

local population. Also, populations might need a certain minimum viable size to allow successful mating and persistence (the so-called Allee effect (Courchamp *et al.* 1999)). Dispersing individuals might be less able to locate small patches, thus decreasing recolonisation chances (Thomas and Jones 1993, Krauss *et al.* 2003b). Many empirical studies indeed show that in small habitat patches species have a larger chance of local extinction and/or a smaller chance of (re)colonisation (Antonovics 1993, Eber and Brandl 1996, Förare and Solbreck 1997, Krauss *et al.* 2003b, Matthies *et al.* 2004).

Second, as the connectivity of patches is reduced species may have to cross larger distances between patches. Also, the surrounding landscape often becomes more difficult to traverse because of different impediments e.g. roads, canals, the lack of available nutrition sources, or detrimental microclimatic conditions, leading to increased mortality during dispersal (Hanski *et al.* 2000, Tischendorf and Fahrig 2000). This reduces the chances of migration between patches and therefore it may lead to a reduction in recolonisations of small and isolated habitat patches (Thomas *et al.* 1992, Bergman and Landin 2001). In addition, small local populations are prevented from being reinforced in numbers or genetic variance that could facilitate persistence (Halley and Dempster 1996) (the so called rescue-effects (Brown and Kodric-Brown 1977)). Furthermore, the decrease in size of surrounding patches may lead to smaller numbers of migrants. In several studies, habitat patches with a low connectivity had a lower chance of being (re)colonised and/or their populations had a higher chance of extinction (Thomas and Jones 1993, Grashof-Bokdam and Geertsema 1998, Kruess and Tscharntke 2000, van Nouhuys and Hanski 2002).

These two effects of fragmentation, a reduced population size and decreased immigration, can lead to inbreeding in habitat patches, which can have detrimental effects on a species (Frankel and Soulé 1981). When inbreeding occurs, species might become more prone to natural enemies, less able to adapt to changes in environmental conditions, and they might experience a decrease in reproductive output (Fischer and Matthies 1997, Ouborg *et al.* 2000, Ivey *et al.* 2004). There is however very limited evidence that inbreeding alone leads to local extinction and some have argued that stochastic effects are probably much more important (Gilpin and Soulé 1986, Menges 1998).

The third consequence of habitat fragmentation is an increase of the edge:interior ratio in the remnant habitat patches. Edges are more prone to environmental influences from outside the patch, often making the patch less suitable for a species to persist (Murcia 1995, Lehtinen *et al.* 2003, Lienert and Fischer 2003). Furthermore, the behaviour of animal species may change near habitat edges (Fagan *et al.* 1999), a.o. affecting migration (Schtickzelle and Baguette 2003) or oviposition rates in insects (Courtney and Courtney 1982, Bellinger *et al.* 1989).

All these effects can make that populations in fragmented habitat patches become more prone to extinction. Studies on occupancy patterns of habitat patches by a species often show strong correlations with patch size, isolation or both (e.g. Hanski *et al.* 1994, Bergman and Landin 2001, Anthes *et al.* 2003). Although there is evidence that some species may adapt to the changing landscape due to selection for a stronger dispersal capacity (Van Dyck and Matthysen 1999), the rate and scale at which human-caused fragmentation occurs is often too large to expect effective adaptation.

Effects of fragmentation on single-species vs. trophic interactions

Traditionally, effects of fragmentation have been considered in the context of direct effects on single species. However, Janzen (1974) already stated that, "The ultimate extinction is the extinction of ecological interactions". Thus, habitat fragmentation can disrupt the assembly and functioning of communities (Wilcove *et al.* 1986, Quinn and Harrison 1988, Baur and Erhardt 1995, Tscharntke *et al.* 2002) because the chance of a particular species persisting in a habitat patch can be strongly affected by the presence or absence of other species. In several studies it has been shown that fragmentation differentially affects the number of species in different guilds and trophic levels, potentially disrupting interactions in entire communities (Kruess and Tscharntke 1994, Zabel and Tscharntke 1998, Golden and Crist 1999, Komonen *et al.* 2000, Kruess and Tscharntke 2000, Tscharntke and Brandl 2004).

The breakdown of community structure in a fragmented habitat can seriously affect flowering plants, whose reproduction depends heavily or completely on the presence and dispersal abilities of their pollinators. In many studies it has been shown that the number of pollinator species decreases in small and isolated patches, leading to a reduction in the number of successful pollinations, which in turn leads to smaller seed set (e.g. Jennersten 1988, Ågren 1996, Steffan-Dewenter and Tscharntke 1999, Schulke and Waser 2001), and potentially to a lower offspring number for the plant in small and isolated plant populations (Groom 2001).

Effects of habitat fragmentation have been shown also in other bi-trophic interactions such as plant-herbivore and plant-pathogen systems (Ouborg and Biere 2003). It is noted that isolated and small patches have a smaller chance of pathogen infection (Antonovics *et al.* 1997, Ericson *et al.* 1999, Groppe *et al.* 2001, Carlsson-Graner and Thrall 2002, Lienert and Fischer 2003, Colling and Matthies 2004) and herbivore occurence (Groom 2001, Kery *et al.* 2001) or a decreased number of herbivore species present (Zabel and Tscharntke 1998, Kruess and Tscharntke 2000). This potentially leads to positive effects of fragmentation on plant performance but to negative effects on herbivore populations. However, it has also been reported that pathogens which become established in smaller fragments are able to infect a larger proportion of individuals within the population (Antonovics 1993, Antonovics *et al.* 1997, Groppe *et al.* 2001, Carlsson-Graner and Thrall 2002). Although the pathogen may not cause direct extinction of plant populations, it results in negative population growth rates and leads to the increased probability of local extinction (Antonovics *et al.* 1997). For herbivores, other studies show that fragmentation may lead to an increase in local herbivore population sizes (Kareiva 1987, Braschler *et al.* 2003) or the level of herbivory (Lienert and Fischer 2003). However, the level of herbivory in patches may be strongly dependent on the presence of the third trophic level, such as predators and parasitoids (see text box 1.1 for an introduction on parasitoids). In this thesis, I focus on the possible consequences of habitat fragmentation for tri-trophic systems consisting of a plant, a herbivore and parasitoids.

Parasitoids in tritrophic interactions

Since the "green world" hypothesis was proposed (Hairston *et al.* 1960) there has been great debate whether parasitoids regulate herbivorous insect populations, thereby decreasing the amount of herbivory (Dempster 1983, Hassell 1986, Hawkins 1994, Rosenheim 1998, Hawkins *et al.* 1999, van der Meijden and Klinkhamer 2000). By killing their herbivorous hosts, it is clear that parasitoids have an effect on the number of adult herbivores of that generation. This could lead to a reduction of herbivores in the next generation if the total number of (larval) offspring is also decreased. In agricultural systems where parasitoids have been released for biological control of pest insects, herbivore numbers and herbivory can be greatly reduced (see Hawkins *et al.* 1999, Hochberg and Ives 2000 for examples).

Whether such a top-down control of parasitoids on host populations is present in natural systems remains to be seen (Dempster 1983, Hassell 1986, Hawkins 1994, Hawkins *et al.* 1999, van der Meijden and Klinkhamer 2000). It is suggested that in natural situations entire parasitoid complexes, rather than single species, control the number of herbivores (Hawkins and Gross 1992, Hawkins 1994). Alternatively, many systems are likely to be controlled by bottom-up processes where the number of herbivores is dependent on the amount and quality of resources supplied through the plants, rather than top-down by predators or parasitoids and that parasitoid numbers therefore depend on the number of herbivore hosts (Price 1994, Roininen *et al.* 1996). Furthermore, in many instances the population dynamics of hosts and parasitoids are greatly influenced by variations in environmental conditions like weather regimes

Text box 1.1 The life history of parasitoids

Parasitoids are insects, whose larvae feed and develop in or on the body of arthropod hosts, eventually killing it (Godfray 1994). They do not move the host to a prepared cache or nest after attack in contrast to several other bee and wasp species. A larva usually develops only on one host individual.

Parasitoids are estimated to constitute up to 20-25 % of all insect species in the world, suggesting that only 5-10 % of 1.6-2 million parasitoid species have been described yet (Godfray 1994). Most parasitoid species belong to the order of Hymenoptera (these are parasitoid wasps). Another important order containing many parasitoids is the Diptera, especially within the large family of Tachinidae (parasitoid flies). Most parasitoids attack other insects, although a few also parasitise other arthropods like spiders, etc. In general, a herbivorous insect species is attacked by a whole complex of parasitoid species. In literature survey, averages of 20 to 70 % parasitism per species are found for different host feeding guilds, caused by an average between 2 and 8 parasitoid species, but parasitoid complexes up to 100 species for a single host species have been found (Hawkins and Lawton 1987, Hawkins 1990, Hochberg and Hawkins 1993, Lewis *et al.* 2002).

One can divide parasitoid species in several classes based primarily on their larval feeding habits (Godfray 1994). I will describe shortly the general properties of the most important types which will be mentioned later in the thesis:

1. **Host stage**. Depending on the host stage on which their larvae are feeding one can group parasitoids into egg parasitoids, larval parasitoids, pupal parasitoids, and adult parasitoids. These parasitoids all attack and develop in the respective stage of the host. Parasitoids that lay eggs in one stage and develop in the next stage, are for example called egg-larval parasitoids or larval-pupal parasitoids.
2. **Larval location**. Parasitoids that feed internally are known as endoparasitoids, which can be further devided into haemolymph feeders, which develop only on the hosts haemolymph and tissue feeders that consume most of the internal bodyparts of the host (Harvey and Strand 2002). Parasitoids that feed externally and imbibe nutrients through skin punctures are called ectoparasitoids.
3. **Clutch size**. Solitary parasitoids usually lay one egg in a host which produces one larva (in contrast to some polyembryonic parasitoids in which multiple larvae hatch from a single egg (Strand 1989)). Gregarious parasitoids lay multiple eggs and many individuals can develop per host.
4. **Host growth**. Parasitoids that allow the host to continue to feed and develop after parasitism are called koinobionts (Askew and Shaw 1986). Those that kill or paralyse and do not allow the host to grow any further after attack are called idiobionts (Askew and Shaw 1986). Egg and pupal parasitoids, as well as those larval parasitoids that paralyse their host permanently upon parasitism, are idiobionts.
5. **Host range**. Depending on the number of host species that a parasitoid can attack, one can distinguish between generalist parasitoids that attack and develop on a wide range of host species and specialist parasitoid species that use only one or a limited number of host species.
6. **Trophic level**. If a parasitoid develops on another parasitoid it is called a hyperparasitoid. A true hyperparasitoid attacks the primary parasitoid when it is still developing in or on its host (the secondary host for the hyperparasitoid); pseudo-hyperparasitoids attack parasitoid larvae when they have already left the host.
7. **Egg maturation**. Furthermore, one can distinguish synovigenic parasitoids that can produce eggs in their ovaries during their life from pro-ovigenic parasitoids that have all eggs already mature and ready to oviposit at the start of adult life (Jervis *et al.* 2001).

(Godfray *et al.* 1994). Another possibility is that parasitoid populations are themselves regulated by a higher trophic level such as hyperparasitoids (Rosenheim 1998, van Nouhuys and Hanski 2000). Currently, many authors agree that in natural communities both bottom-up and top-down regulation play a role simultaneously (Hunter and Price 1992).

In addition to the effects that parasitoids may have on the population dynamics of the host and therefore on herbivory, most parasitoids have a direct effect on herbivory by reducing the quantity of food consumed by an individual parasitised host. It is clear that egg-parasitoids prevent any feeding of the host and also larval idiobionts stop feeding immediately upon attack by killing or paralysing the host (see Text box 1.1 for an overview of different life history characters of parasitoids). Also most koinobionts, especially solitary ones, cause a decrease in food consumption by the host (Huebner and Chiang 1982, Rohlfs and Mack 1983, Schopf and Steinberger 1996).

However, not all parasitoids have this effect and a plant does not always directly benefit from parasitism (van der Meijden and Klinkhamer 2000). Pupal, larval-pupal and larval parasitoids that attack fully grown larvae, will not have a direct effect on herbivory because the herbivore is parasitised after it has completed feeding. Gregarious koinobionts inject biochemical factors into the host during oviposition that regulate host growth and behaviour to such an extent that consumption can be similar or even increased compared to healthy hosts (Rahman 1970, Slansky 1978, Beach and Todd 1986). In many gregarious species host growth is positively correlated with the number of larvae developing in a host and in some species only large numbers of larvae lead to an increase in host growth and consumption that is above the level of healthy hosts (Sato and Tanaka 1984, Harvey 2000).

Habitat fragmentation and plant-herbivore-parasitoid interactions

One result of habitat fragmentation is a reduction in the number of herbivorous species present in small or isolated plant habitat patches (Zabel and Tscharntke 1998, Kruess and Tscharntke 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 Tscharntke 1998).

However, a number of studies suggest that also parasitoid species are frequently absent in small and isolated patches, leading to a reduction in parasitism in isolated and small habitat patches (Kruess and Tscharntke 1994, Roland and Taylor 1997, Kruess and Tscharntke 2000). 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 individual insect larvae (Guillot and Vinson 1973, Powell 1989, Elzinga *et al.* 2003). If herbivores can escape from their natural enemies, theoretically they can overexploit their food plant, which could lead to instability of the whole system. Eventually this may lead to increased extinction risks of all the trophic levels.

Several studies suggest that the third trophic level is more strongly affected by habitat fragmentation than the second trophic level (Kareiva 1987, Komonen *et al.* 2000, Tscharntke and Brandl 2004). One of the explanations is that predators and parasitoids have lower dispersal abilities than their hosts and that isolated plant patches are more easily colonised by herbivorous insects than by their enemies, even at scales of tens of meters (Kruess and Tscharntke 1994, Zabel and Tscharntke 1998, Komonen *et al.* 2000, Kruess and Tscharntke 2000). However, whereas for many herbivores there is at least some knowledge of their dispersal abilities, knowledge of dispersal abilities of parasitoids is scarce. In contrast to the community studies where effects of fragmentation were already found at scales less than a few hundred meters (Kruess and Tscharntke 1994, 2000), many studies on individual species suggest that parasitoid species can travel several kilometres and that sometimes their dispersal abilities even exceeds that of their host (Antolin and Strong 1987, Godfray 1994, Dempster *et al.* 1995a, Dempster *et al.* 1995b, Jones *et al.* 1996, Goldson *et al.* 1999, van Nouhuys and Hanski 2002). A further explanation for the differences in the effects of fragmentation on herbivores and parasitoids may be that parasitoid populations are more vulnerable to extinctions due to smaller and more variable population sizes (Kruess and Tscharntke 1994, 2000, van Nouhuys and Tay 2001).

Habitat fragmentation may not only alter the distribution patterns of species but also change the behaviour of species. Several studies have shown that dispersal behaviour can be altered by patch size, resulting in an increase in the number of individuals dispersing from smaller patches (Sheehan and Shelton 1989, Bergman and Landin 2001). Also, a larger proportion of individuals may stay or return in fragmented patches (Baguette *et al.* 2003). Differences in migration rate will obviously affect the number of individuals in a patch and therefore the level of herbivory or parasitism rate. Oviposition rate of insects may also be affected by the size of plant patches (Maguire 1983, Shea *et al.* 2000), leading to differences in the level of herbivory or parasitism. An increase in travelling time between patches can also be expected to change oviposition rates of herbivores as well as of parasitoids.

Whereas the decrease in size and the increase in isolation are recognised as important factors leading to changes in herbivory level, the effects of changes in edge:interior ratio are less well studied (Fagan *et al.* 1999). Biotic interactions in edges can be different from those in the centre of a patch, leading to differences in oviposition levels (Courtney and Courtney 1982, Cappucino and Martin 1997), herbivory (Lienert and Fischer 2003) or parasitism rates (Roland 1993, Cronin 2003b).

Scope and aim of the thesis

The general aim of this thesis is to study the effects that habitat fragmentation has on a plant-herbivore-parasitoid-system. Until now, most studies on interactions in experimental or natural plant patches with different degrees of fragmentation either focused on plant-herbivore interactions (Groom 2001, Lienert and Fischer 2003, Colling and Matthies 2004) or herbivore-parasitoid interactions (Doak 2000, van Nouhuys and Tay 2001, van Nouhuys and Hanski 2002). In this thesis I investigate whether any effects of habitat fragmentation (at a landscape scale) on the parasitism rate of herbivores has direct consequences for the level of herbivory of a plant species. More specifically, I test the hypothesis that the presence of parasitoids is decreased more strongly by habitat fragmentation than the presence of their herbivorous host and whether this has negative effects on the plant due to increased herbivory.

In this thesis I use a model system of a fairly common plant species which occurs in distinct patches, a specialist herbivore and the parasitoids that attack the herbivore. By studying the natural occurrence patterns of the herbivore and its parasitoid species in combination with the herbivory level and the level of parasitism in plant patches that differ in degree of fragmentation, I draw conclusions about the effect that fragmentation may have on this model system. With a colonisation experiment I investigate the dispersal abilities of the different species. In an experimental study I test whether edge-effects can play a role in herbivore and parasitoid oviposition behaviour leading to differences in herbivory level or parasitism rate. Finally, with laboratory experiments I investigate more specifically the interaction between host and parasitoid in particular, the potential effect that parasitoids might have on herbivory in this system, and whether oviposition decisions by parasitoids might change depending on their internal state. The specific outline of the thesis is presented at the end of this chapter.

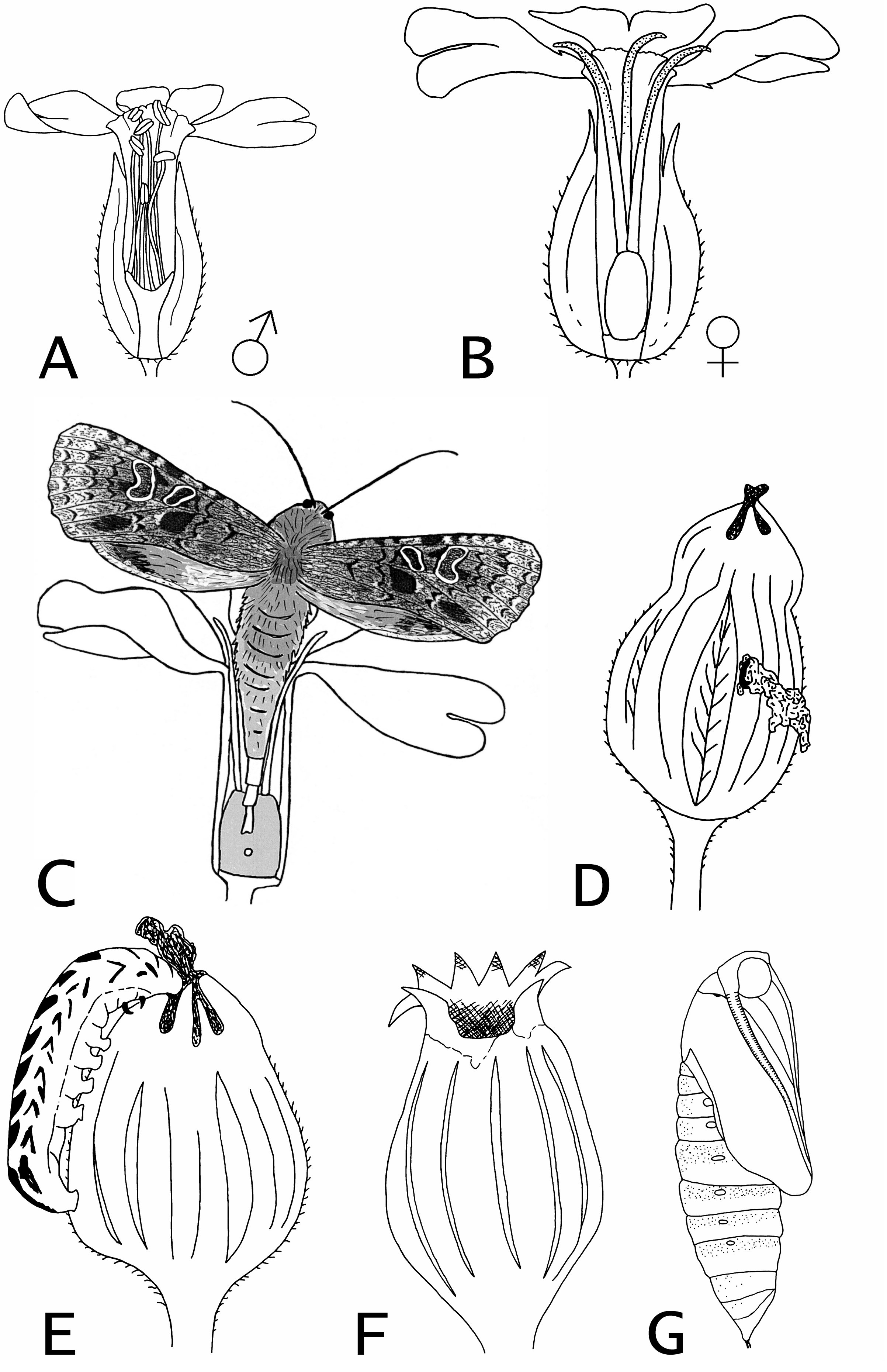
Study system

The plant

In this study I use the white campion, *Silene latifolia* Poiret 1789 (= *S. alba* (Miller) Krause = *S. pratensis* (Rafn.) Godr. & Gren. = *Lychnis alba* Miller = *L. vespertina* Sibthorp = *Melandrium album* (Miller) Garcke). It is a weedy short lived perennial occurring in open disturbed habitats such as fallow fields, field margins and roadsides (Goulson and Jerrim 1997) predominantly on chalky sandy soil (Baker 1947). It is native to Eurasia, and has been introduced to North America where it is considered a pest species (Wolfe 2002). It is one of the plants that has spread with agriculture in north-western Europe (Mastenbroek 1983).

S. latifolia is a dioecious plant, which means that there are male plants, producing male staminate flowers (Fig. 1.2 A), and female plants, producing female pistillate flowers (Fig 1.2 B) (Geber *et al.* 1999). Male and female plants differ in biomass allocation to flowers, flower phenology, flower size and flower number (Gross and Soule 1981, Grant *et al.* 1994, Carroll and Delph 1996, Meagher and Delph 2001, Delph *et al.* 2002). The sex ratio observed in natural populations is often female biased, mainly caused by differences in survival and longevity between the sexes (Geber *et al.* 1999, but see Taylor 1999 for a genetic explanation). The sex determining system is similar to that in many animals where the presence of a Y chromosome leads to the development of male characteristics (Warmke 1946). It is now a model species for studies on many ecological, evolutionary and genetical aspects of sexual dimorphism (Geber *et al.* 1999).

In north-western Europe *S. latifolia* has a flowering time from the end of April up to the end of October but it usually has two distinct flowering peaks at the end of May and the beginning of August (Biere and Honders 1996a, Jürgens *et al.* 1996, Bopp 2003). Its flowers have white petals that usually open in the late afternoon and close again late in the morning the next day. Male flowers are open for 1-2 days whereas unpollinated female flowers remain open for up to 7 days before wilting. Upon fertilisation, the female flowers wilt within a few hours. During the night flowers emit a strong scent attracting noctuid moths and hawkmoths (Jürgens *et al.* 2002a). Pollination in the Netherlands is mainly performed by moths, with *Hadena bicruris* and *Autographa gamma* as most common visitors, although some hoverfly species visit the flowers during the day (van Putten *et al.* 2002). Also in America several insect species visit the flowers (Altizer *et al.* 1998) but moths seem to be the most important pollinators (Young 2002). After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successful fertilisation the ovule expands and develops into a seed capsule containing several hundreds of seeds (Jürgens *et al.* 1996). Natural seed dispersal distance is probably very limited. Young (2002) found seeds dispersing on average only 40 cm and also genetic studies suggest that seed movement is limited (McCauley 1997). This may be one of the reasons why *S. latifolia* grows in distinct patches. Long distance dispersal of the plant is probably strongly influenced through soil or vegetation movement by humans.

S. latifolia has several natural enemies. Next to generalists like some rust fungi, slugs and insect species (Baker 1947, Keller *et al.* 1999), several specialists can be found commonly on *S. latifolia* in the Netherlands (Wolfe *et al.* 2004). Two of these affect the reproduction of the species directly.

First, the anther smut *Microbotryum violaceum* is a heterobasidiomycete fungus that sterilises several species of Caryophyllaceae. One host race of this fungus is known from *S. latifolia* (van Putten 2002). When a plant has become systemically infected all flowers that are produced are sterile, producing only fungal spores in the anthers. The fungus induces a morphological sex change in female flowers (Ruddat *et al.* 1991), initiating the production of spore-bearing anthers. In female flowers of infected plants only a remnant of an ovule can be found. The spores are dispersed to flowers of healthy plants by several pollinators (Altizer *et al.* 1998). The spatial ecology of the interaction has been studied quite intensively in the United States (Antonovics 1993, Antonovics *et al.* 1997). These studies indicate that colonisation probability of the pathogen increases with increasing connectivity and plant patch size (Antonovics *et al.* 1997), probably partly as a result of decreased dispersal of vector insects between patches (Roche *et al.* 1995).

The most important herbivore, on which this thesis focuses, is the noctuid moth *Hadena bicruris*, whose larvae consume the developing seeds.

The herbivore

Hadena (Schrank, 1802) species are specialist Lepidoptera (Noctuidae) on plants in the pink family Caryophyllaceae. For an extensive overview of the *Hadena* family see Hacker (1996). The lychnis, *Hadena bicruris* Hufnagel 1766 (*Phalaena* Hufn.)

Figure 1.2 (opposite page) The life cycle of *Hadena bicruris* on *Silene latifolia*. (A) male flower. (B) female flower. (C) female *H. bicruris* with extended ovipositor depositing an egg on the ovary of a female flower. (D) Primary seed capsule containing an instar 3 caterpillar with excrements protruding from the entrance hole. (E) Instar 5 caterpillar attacking a (secondary) seed capsule with its head inserted in the fruit. (F) Typical bite marks of a *H. bicruris* caterpillar on a secondary seed capsule. (G) Pupa of *H. bicruris*.

(*Dianthoecia* B.) (*Harmodia* Hbn.) (*capsincola* Schiff.) (*capsincoloides* Standfuss) has a very strong preference for *S. latifolia* (Wirooks and Plassmann 1999, Bopp 2003, Dötterl 2004). Other plant species on which it has been recorded less often are *S. vulgaris*, *S. dioica* and rarely *Saponaria officinalis* (Steiner and Ebert 1998, Wirooks and Plassmann 1999, Bopp and Gottsberger 2004, Dötterl 2004). *H. bicruris* is a night-active moth with a wingspan of 30-40 mm and it has a characteristic long ovipositor and a long proboscis as an adaptation to the deep corolla of *S. latifolia* flowers (see for a further habitus description Bretherton *et al.* 1979). Recently, it has been suggested to split the species into *H. bicruris* that is common in western Europe and *H. capsincola* Deniz & Schiffermüller 1775 that seems to replace the former in eastern and northern Europe (Hacker 1996). In western Europe *H. bicruris* is found in more than 90 % of *S. latifolia* populations (Wolfe 2002) where it destroys 25 - 100 % of all seed capsules, apparently having a major impact on the fitness of the plants (Biere and Honders 1996b, Wolfe 2002).

After nectar feeding on a female flower of *S. latifolia*, a female moth can decide to oviposit. It will extend its ovipositor and deposit one egg, usually on the ovule (Fig. 1.2 C) but occasionally on other parts of the flower (Brantjes 1976b). Although in captivity it will oviposit many times in one flower (Elzinga *et al.* 2002), it can recognise flowers with already an egg present and prevent superparasitism under more natural circumstances (Brantjes 1976a). Directly after hatching, the first instar caterpillar (L1) chews a small hole in the ovary, enters the young fruit, and starts to feed on the ovules and developing seeds. Through the entrance hole red frass is excreted (Fig. 1.2 D), which is a clear sign that a seed capsule contains a developing larva. It will stay in this so-called primary seed capsule until it has consumed all the seeds, which usually occurs as a fourth or final fifth instar (hereafter L4 and L5, respectively). At this stage the caterpillar leaves the primary capsule and starts feeding on other seed capsules (so-called secondary seed capsules). The caterpillar first chews a large hole in the top of these seed capsules and than progresses to the seeds (Fig. 1.2 E). Of capsules that have already opened but have not yet shed seeds it bites several sharp capsule teeth away before entering the fruit (Fig. 1.2 F). Late instar caterpillars are usually too large to be self-contained within these secondary seed capsules, and thus the caudal appendages usually protrude from the capsule during feeding bouts (Brantjes 1976b, Steiner and Ebert 1998, Biere *et al.* 2002). Many large caterpillars will also hide in empty seed capsules during the day. At maturity, on average about 4 weeks after hatching, the caterpillar leaves the food plant and pupates in the soil in a loosely spun cocoon. Depending on the time of year the moths emerge after approximately three weeks or the pupa (Fig. 1.2 G) enters diapause and develops after the winter (Elzinga *et al.* 2002).

Adults fly from mid-May until the beginning of September, in probably two overlapping generations. Although clearly there are two distinct peaks in oviposition in early June and August coinciding with the peaks in flowering of *S. latifolia* (Biere and Honders 1996b), eggs are deposited on flowers throughout the whole season (Bopp 2003). Caterpillars can be found from the end of May until late October (Brantjes 1976b).

The relationship between *S. latifolia* and *H. bicruris* has sometimes been referred to as a mutualistic relationship (Olberg 1951, Bopp 2003). The moth benefits the plant by pollinating its flowers, while the plant supplies in return food to the larvae of the moth. Similar relationships between plants and frugivorous insects can be found, with very good examples for figs and fig wasps (Janzen 1979), yucca and yucca moths (Pellmyr 2003) and *Trollius* with Chiastochaeta (Pellmyr 1992). However, several studies have made clear that *H. bicruris* is to be considered as a parasite of the plant rather than a mutualist. Based on the nectar needs of the moth and the nectar content in a *S. latifolia* flower, a pair of moths will maximally pollinate 500 female flowers during a lifespan of two weeks (Brantjes 1976b, Bopp 2003). In contrast, a female moth produces 100-400 eggs during her life (Brantjes 1976b, Elzinga *et al.* 2002, Bopp 2003), and each larva can destroy about 5-7 capsules during its development (Brantjes 1976b, Bopp 2003). This leads to the conclusion that the offspring causes more damage than the plant profits from pollination by one pair of *H. bicruris* and therefore a true mutualism does not exist between these two species. Also, the two species do not strictly depend on each other. *H. bicruris* uses other caryophyllaceous plant species as a nectar source (Jürgens *et al.* 1996) and, *S. latifolia* is not solely dependent on *H. bicruris* for its pollination. Several other moth species and even diurnal pollinators can pollinate the flowers (Jürgens *et al.* 1996, 2002b, van Putten 2002). Petterson (1991) shows that *H. bicruris* is actually a relatively ineffective pollinator on *S. vulgaris*. In America, due to its absence, *H. bicruris* does not pollinate *S. latifolia,* but still the plant is pollinated and reproduces there to such an extent that it is considered a pest species in several regions (Wolfe 2002, Young 2002).

The parasitoids

Several studies have mentioned that parasitoids may cause significant mortality of *H. bicruris* caterpillars (Brantjes 1976b, Biere *et al.* 2002), and may consequently reduce the level of seed predation by *H. bicruris* even to such an extent that the relation between *S.*

latifolia and *H. bicruris* could become mutualistic (Bopp 2003). However, none of these studies so far have described and quantified in detail which species of parasitoids were responsible for the parasitism. Two hymenopteran species were observed regularly previous to the research presented in this thesis (Biere, pers. comm.); *Microplitis tristis* (Braconidae), a specialist koinobiont gregarious endoparasitoid and *Bracon variator* (Braconidae) a generalist gregarious ectoparasitoid.

The study area

The main area where the study of this thesis was performed is 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 near the city of Gorinchem. The area consists of floodplains surrounded by river dikes. While a large fraction of the area is used for nature conservation where only herds of horses and cattle graze, other parts are used for agriculture (mainly haymaking) or for industrial purposes (e.g. brick factories). *S. latifolia* patches mainly occur on the elevated sandy places, but also in disturbed areas near industrial sites and along roadsides and field margins. Many patches of *S. latifolia* also grow on the river dikes, which are mostly mown twice a year. The surrounding area is mostly unsuitable for *S. latifolia* due to heavy agriculture and less suitable soil. Due to its linear structure and its open nature, the area can be searched effectively for *S. latifolia*, also because the plants are easily located during their peak flowering period. The connectivity of every plant patch can be reliably determined, because the location and size of all surrounding patches are known. Due to the differences in land management, patches of *S. latifolia* with different degrees of fragmentation occur, which makes the area ideal for this study.

Outline of the thesis

To study what effects habitat fragmentation might have on a plant-herbivore-parasitoid system it is essential to know by which parasitoid species the herbivore is attacked and to what extent. Although two species were known that attack the host, nothing is known about other parasitoid species nor is any quantitative information about parasitism rates available. In **chapter 2** therefore I quantify to what extent *H. bicruris* is parasitised and by which species. I will give a detailed description of the life history of all parasitoid species in the parasitoid complex associated with *H. bicruris* to discuss whether they might have a direct impact on the level of herbivory of individual caterpillars.

This important aspect is further investigated in **chapter 3**. Here, I study the effect that parasitism by the most numerous parasitoid, the specialist gregarious *M. tristis*, has on the food consumption by the host. Furthermore, I determine how the parasitoid is adapted to variation in host size at oviposition by measuring several fitness estimates such as offspring weight and survival. It is expected that the parasitoid can adapt to different host sizes by changing clutch size, development time or host food consumption.

In **chapter 4** I present data on the presence of the three most important parasitoids and the herbivore in natural populations of the plant differing in connectivity and size. I test whether the presences of these parasitoids, the specialist gregarious endoparasitoid *M. tristis*, the generalist gregarious ectoparasitoid *B. variator* and the specialist solitary larval-pupal parasitoid *Eurylabus tristis,* are related to isolation and size of the plant patches. To investigate whether differences in distribution patterns could be caused by different dispersal abilities, I measure colonisation rates in experimental plant patches located at distances of 125-2000 m from possible natural source populations. I discuss why certain species of parasitoids might be differentially affected by habitat fragmentation.

Whether the differences in distribution patterns of the parasitoids also result in differences in the level of herbivory is investigated in **chapter 5**. I show the relation between the level of fragmentation of the natural habitat patches and the level of herbivory by *H. bicruris* on *S. latifolia* and the parasitism rate. Because herbivory may not only be affected by parasitism rate but also by the relative number of eggs deposited by the herbivore I conduct an experiment to show potential differences in oviposition rates in patches of different sizes. Furthermore, I investigate in patches of controlled size and structure whether edge-effects on oviposition behaviour may play a role in causing a correlation between patch size and the level of herbivory.

Because travelling to isolated patches costs time for a parasitoid in **chapter 6** I explore the effects that age or an increased time to the first host encounter might have on the clutch sizes that the most important gregarious parasitoid in the system *M. tristis* produces. I test to what extent models that predict how a synovigenic parasitoid should optimally distribute its eggs over time, are applicable to *M. tristis*.

Finally, in **chapter 7** I discuss the patterns that are found and how habitat fragmentation may have an effect on this system. I conclude with some general remarks about what this study has learned us about the consequences of habitat fragmentation.

Chapter 2

The parasitoid complex associated with the specialist *Hadena bicruris* (Lepidoptera: Noctuidae) on *Silene latifolia* (Caryophyllaceae) in the Netherlands along the river Waal

with Kees Zwakhals, Jeffrey A. Harvey and Arjen Biere

Abstract

***Hadena bicruris* is the most important predispersal seed predator of *Silene latifolia*. Parasitoids attacking the larvae of this specialist noctuid can potentially decrease the amount of damage to the plant. However, the parasitoid complex associated with *H. bicruris* has not yet been described nor quantified in detail. This paper describes and quantifies the parasitoid complex associated with *H. bicruris* along the river Waal in the Netherlands, and gives information about life history characters of the individual parasitoid species.**

From a total of 1773 collected caterpillars, 44 % were parasitised by at least 14 species of parasitoids. The most important parasitoids were the braconids *Microplitis tristis* (22.9%) and *Bracon variator* (4.9%) and the ichneumonids *Eurylabus tristis* (11.7%) and *Ophion pteridis* (3.4%). Other species occurring sporadically were: the ichneumonids *Scambus brevicornis, S. buolianae, S. annulatus, Erigorgus cerinops,* and *Hyposoter* sp.; the tachinids *Phryxe vulgaris*, *P. nemea, Blondelia nigripes* and *Siphona geniculata*; and a Mermithidae sp. On *M. tristis* the ichneumonid true hyper-parasitoid *Mesochorus lanceolatus* was occasionally found. The ichneumonid pseudohyper-parasitoids *Gelis agilis*, *G. hortensis* and the chalcids *Baryscapus endemus*, *Pteromalus chrysos* and *P. vibulenus* were found on cocoons of both *M. tristis* and *B. variator*. Only *M. tristis*, *E. tristis* and *M. lanceolatus* are specialists on their hosts. All others are generalists attacking many other species.

The effect of the parasitoids on herbivory by their host is probably small because most of the parasitoid species are koinobionts that attack large hosts. Only *B. variator*, which is an ecto-parasitoid, stops host development immediately upon parasitism, but like the other parasitoids, it attacks mainly large L4 or L5 hosts.

The gregarious *M. tristis* and *B. variator* produce clutches with a female biased sex ratio, in contrast to the solitary *E. tristis* where the sex ratio approaches equality. The ectoparasitoid *B. variator* produces mostly single-sex clutches, probably avoiding deleterious effects of inbreeding. Large clutches of *M. tristis* and *B. variator* are probably caused by superparasitism.

Introduction

Caterpillars of the specialist noctuid moth *Hadena bicruris* are the most important predispersal seed-predators of *Silene latifolia* (Biere and Honders 1996b). In western Europe it is found in more than 90 % of *S. latifolia* populations (Wolfe 2002) where it destroys 25 - 100 % of all seed capsules, apparently having a major impact on the fitness of the plants (Biere and Honders 1996b, Wolfe 2002). Although the adult *H. bicruris* is also an important pollinator of *S. latifolia*, the number of seed-capsules damaged by the offspring of a moth outnumbers the possible positive effects of pollination (Brantjes 1976b, Bopp 2003).

Several studies have suggested that parasitoids may cause significant mortality of *H. bicruris* caterpillars, and may consequently reduce the level of seed predation (Brantjes 1976b, Biere *et al.* 2002, Bopp 2003). However, none of these studies so far have described and quantified in detail which species of parasitoids were responsible for the parasitism. Although taxonomic works on parasitoids (Shenefelt 1980, Brock 1982, Belshaw 1993) and databases like Taxapad (Yu 1998, Noyes 2001) indicate that several species develop on *H. bicruris*, these data are, for parasitoids in general, rather scattered, incomplete and may partly be based on erroneous records (Shaw 1994). Furthermore, we have found almost no literature reporting hyperparasitoids in this host-parasitoid complex (except for Schwarz and Shaw 1999). In addition, of the many parasitoid species identified thus far, knowledge on life history parameters like clutch size, sex ratio, host stage attacked, etc., under natural conditions is very limited, although it can provide valuable insights into the strategies that different parasitoids employ to exploit suitable hosts in their habitat (Godfray 1994).

The first aim of this paper is to describe and quantify the parasitoid complex, including several hyperparasitoids, associated with the specialist noctuid host, *H. bicruris* on *S. latifolia*. We will discuss the level of specialisation on *H. bicruris* for the different parasitoid species. The second aim is to provide detailed information of the biology of the observed parasitoids. Based on this, we will discuss whether the parasitoids in the system may contribute to a more mutualistic plant-host relation between *H. bicruris* and *S. latifolia*. Finally, we will discuss the different patterns in clutch size and sex ratio observed for the most important species of parasitoids in the system.

Methods

Host

Hadena bicruris Hufnagel 1766 (*Phalaena* Hufn.) (*Dianthoecia* B.) (*Harmodia* Hbn.) (*capsincola* Schiff.) (*capsincoloides* Standfuss) (Lepidoptera: Noctuidae) is, like other members from its family (see Hacker (1996) for a revision of the family), specialised on the seed capsules of Caryophyllaceous plant species, but has a very strong preference for *Silene latifolia* Poiret 1789 (= *S. alba* (Miller) Krause = *S. pratensis* (Rafn.) Godr. & Gren. = *Lychnis alba* Miller = *L. vespertina* Sibthorp = *Melandrium album* (Miller) Garcke) (Wirooks and Plassmann 1999, Bopp 2003). Recently, it has been suggested to split the species into *H. bicruris* that is common in western Europe and *H. capsincola* Denis & Schiffermüller 1775 that seems to replace the former in eastern and northern Europe (Hacker 1996).

After nectar feeding and pollination of *S. latifolia*, a female moth can decide to oviposit in the female flower. It will extend its ovipositor and deposit an egg, usually on the ovule but occasionally on other parts of the flower (Brantjes 1976b). Directly after hatching the first instar caterpillar (L1) chews a small hole in the ovary, enters the young fruit, and starts to feed on the ovules and developing seeds. It will stay in this so-called primary seed capsule until it has consumed all the seeds, which usually occurs as a fourth or final fifth (hereafter L4 and L5, respectively) instar. At this stage the caterpillar leaves the primary capsule and starts feeding on other (secondary) seed capsules. The caterpillar first chews a large hole in the top of the secondary seed capsule and than progresses to the seeds. Of capsules that have already opened but have not yet shed seeds it chews away several sharp capsule teeth before entering the fruit. Late instar caterpillars are usually too large to be self-contained within these seed capsules, and thus the caudal appendages usually protrude from the capsule during feeding bouts (Biere *et al.* 2002). Many large caterpillars will also hide in empty seed capsules during the day. At maturity, on average about 4 weeks after hatching, the caterpillar leaves the food plant and pupates in the soil. Depending on the time of year the moths emerge after approximately three weeks or the pupa enters diapause and develops after the winter (Elzinga *et al.* 2002).

Adults fly from the middle of May until the start of September, in usually two overlapping generations. Although clearly there are two distinct peaks in oviposition in early June and August coinciding with the peaks in flowering of *S. latifolia* (Biere and Honders 1996b), eggs are deposited on flowers throughout the season (Bopp 2003).

Study area

To quantify the parasitoid complex associated with *H. bicruris* and to describe life history parameters, we performed a study along 100 km of the rivers Rhine and Waal (main branch after splitting of the Rhine) in the Netherlands from the German border up to the city of Gorinchem. In this area we located all *S. latifolia* patches, 86 in total, ranging in size from 1 to circa 12.000 plants (Chapter 4 and 5).

Patches of *S. latifolia* occur mainly on river dikes and the higher sandy parts of floodplains, but also in disturbed areas near (brick) factory sites. In many places the vegetation is mown twice a year resulting in grasslands with low vegetation height, but other patches are located in nature reserves with cattle grazing, leading to a more rough and taller vegetation. In general, *S. latifolia* seems to prefer open habitats through mowing, grazing and other disturbances. In addition to these natural plant patches in the study area some observations were made in the experimental garden of our institute in Heteren, The Netherlands, at 25 km from the study area.

Sampling and rearing methods

Over the course of three years, 2001, 2002 and 2003, we visited the plant patches during the peak flowering periods, i.e. June and August and a few weeks later when most seed capsules were ripe. During these visits we collected all visible caterpillars, i.e. caterpillars not present in primary seed capsules, but on the plant or (partly) hiding in secondary seed capsules. Living caterpillars from inside primary seed capsules were never found being parasitised and were thus not considered (Elzinga, unpub. data). However, occasionally we were also able to collect parasitoid larvae or cocoons on dead caterpillars in primary and secondary seed capsules. Additional caterpillars and immature parasitoids were collected from several hundreds of *S. latifolia* plants located in the garden in 2003.

All collected caterpillars and immature parasitoids were taken to the laboratory and put in separate vials. The instar stage of every caterpillar was determined by examining the headcapsule dimensions (not possible for all of the dead caterpillars) and it was further assessed whether they were alive, dead or paralysed, and/or parasitised by parasitoids. If caterpillars were alive, a small block of artificial diet was added to the vial (Elzinga *et al.* 2002). When caterpillars neared the end of the fifth stadium, the vial was half filled with vermiculite to allow pupation or egression of parasitoids.

The number of parasitoid larvae that developed from a host was counted. All dead caterpillars were dissected to assess the presence of immature parasitoid larvae. If the parasitoid adults successfully emerged from their cocoons, their sex was determined.

All rearing was done at 25/20 ºC and 16/8 h light/dark at 70 % humidity except for caterpillars collected in late summer. These were reared at 20/15 ºC and 8/16 h light/dark to initiate diapause (further referred to as autumn conditions). After 6-8 months at 4 ºC and 8/16 h light/dark and 70 % humidity, the diapaused moth pupae and parasitoid cocoons were placed at 25/15 ºC and 16/8 h light/dark to break the diapause.

Identification

Specimens of adult parasitoids were stored and identified by several specialists: Prof. Dr. C. van Achterberg (Braconidae), M.J. Gijswijt (Chalcidoidae), Dr. M. Schwarz (*Gelis* sp.), L.E.N. Sijstermans (Tachinidae) and Kees Zwakhals (Ichneumonidae).

Analyses

For the quantification of the parasitoid complex we used only data gathered from caterpillars collected in the natural *S. latifolia* patches (hereafter referred to as the field). For rare species, hyperparasitoids and for life history characteristics we used additional data from caterpillars collected in experimental plant patches near the Waal and from the experimental garden. Sex ratios were determined by counting the total numbers of males and females that had emerged. Correlations between sex ratio and clutch size were analysed with generalised linear models with a quasibinomial distribution and a logit link function (GLM) (McCullagh and Nelder 1989) in the statistics computerprogramme R (Venables *et al.* 2003).

Results

Primary parasitoids

In total we were able to collect 1773 caterpillars from the field, mainly live larvae as L5 (Table 2.1). Of these, 44.2 % were parasitised by various parasitoid species, 44.8 % either developed into a moth or died as a pupa with no signs of parasitism, and 11.0 % of all caterpillars were dead or died as a caterpillar without indications of parasitism (Fig. 2.1, Table 2.2).

Table 2.1 The number and status of *H. bicruris* caterpillars collected from natural *S. latifolia* patches.

	2001		2002		2003	
	nr.	%	nr.	%	nr.	%
Total	553		528		692	
Alive	493	89.2	493	93.4	635	91.8
Dead	60	10.8	35	6.6	57	8.2
Instar 3	17	3.1	6	1.1	5	0.7
Instar 4	136	24.6	79	15.0	81	11.7
Instar 5	371	67.1	432	81.8	589	85.1
Instar ?	29	5.2	11	2.1	17	2.5

Caterpillars were parasitised by at least 14 different species of parasitoids (Table 2.2). Although some differences in the level of parasitism per species were observed between years, the overall pattern was relatively constant (Fig. 2.1). In total we could identify eight hymenopteran primary parasitoids associated with *H. bicruris* along the river Waal at the species level: two gregarious braconid species and six solitary ichneumonid species. A very small percentage of caterpillars (less than 1%) was parasitised by at least four tachinid (Diptera) species. All common species appeared to be present almost during the entire flowering season of *S. latifolia*, the food plant of *H. bicruris* (Fig. 2.2). Below we present more detailed information per species.

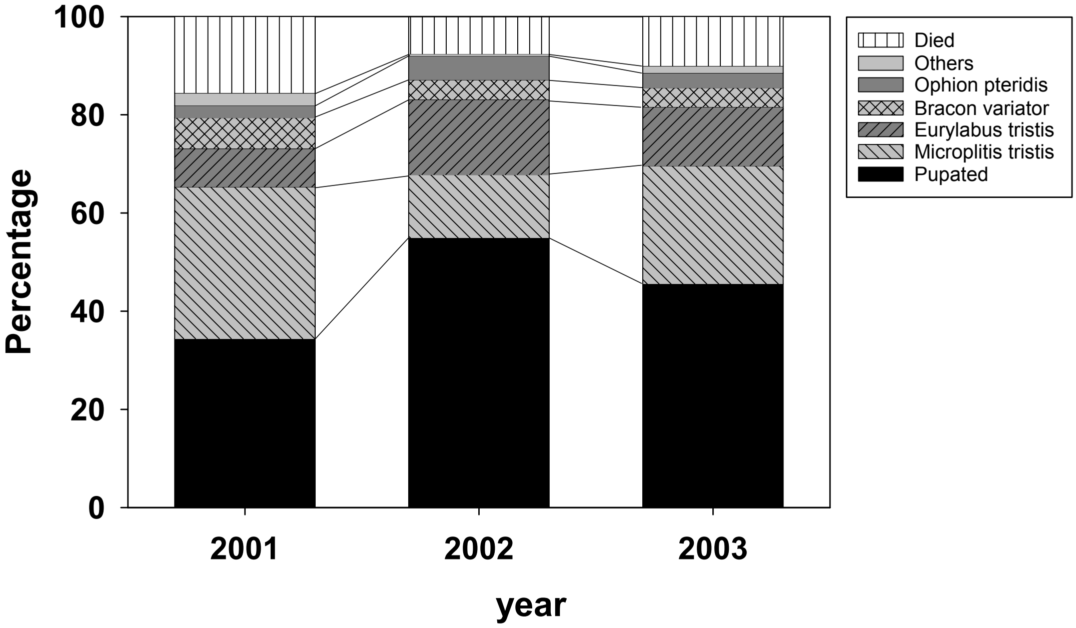
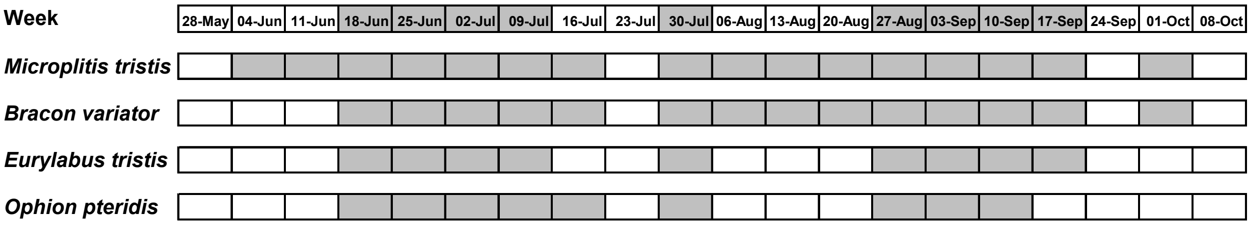


Figure 2.1 The fate of all *H. bicruris* caterpillars collected in natural *S. latifolia* patches. Only the parasitism rate of the most common species are shown individually, whereas the others are grouped. Caterpillars that were collected dead or which died without signs of parasitism are assigned to the group Died.



Week	28-May	04-Jun	11-Jun	18-Jun ▨	25-Jun ▨	02-Jul ▨	09-Jul ▨	16-Jul	23-Jul	30-Jul ▨	06-Aug	13-Aug	20-Aug	27-Aug ▨	03-Sep ▨	10-Sep ▨	17-Sep ▨	24-Sep	01-Oct	08-Oct
Microplitis tristis		▨	▨	▨	▨	▨	▨	▨		▨	▨	▨	▨	▨	▨	▨	▨		▨	
Bracon variator				▨	▨	▨	▨	▨		▨	▨	▨	▨	▨	▨	▨	▨		▨	
Eurylabus tristis				▨	▨	▨	▨			▨				▨	▨	▨	▨			
Ophion pteridis				▨	▨	▨	▨	▨		▨				▨	▨	▨				

Figure 2.2 The periods (darkened bar sections) in which larvae of the most common parasitoid species of *H. bicruris* were found. During the weeks that are darkened we collected most caterpillars.

Table 2.2 The fate of all *H. bicruris* caterpillars collected in natural *S. latifolia* patches, including those that were dead when collected. Between brackets parasitoids that developed after *M. tristis* had emerged.

	2001		2002		2003	
	nr.	%	nr.	%	nr.	%
Total Hymenoptera	269	48.6	199	37.7	303	43.8
Microplitis tristis	171	30.9	68	12.9	167	24.1
Eurylabus tristis	43	7.8	81	15.3	83	12.0
Bracon variator	35(1)	6.3	21	4.0	27(3)	3.9
*Bracon brevicornis**						
Ophion pteridis	14	2.5	26	4.9	21(1)	3.0
Scambus sp. total	6	1.1	2	0.4	2	0.3
Scambus brevicornis	2	0.4	1	0.2	1(1)	0.1
Scambus buolianae	1	0.2				
Scambus annulatus					1	0.1
Erigorgus cerinops					2	0.3
Hyposoter sp.					1	0.1
Unidentified	2	0.4				
Total Tachinidae	5	0.9			6	0.9
Blondelia nigripes					2	0.3
Siphona geniculata					1	0.1
Phryxe vulgaris					2	0.3
Phryxe nemea					1	0.1
Unidentified	5	0.9	(1)			
Nematodes sp.	1	0.2			(1)	
Pupated	190	34.4	290	54.9	314	45.4
Dead (no sign of parasitism)	86	15.6	40	7.6	69	10.1

*Developed once on a host collected in the experimental garden.

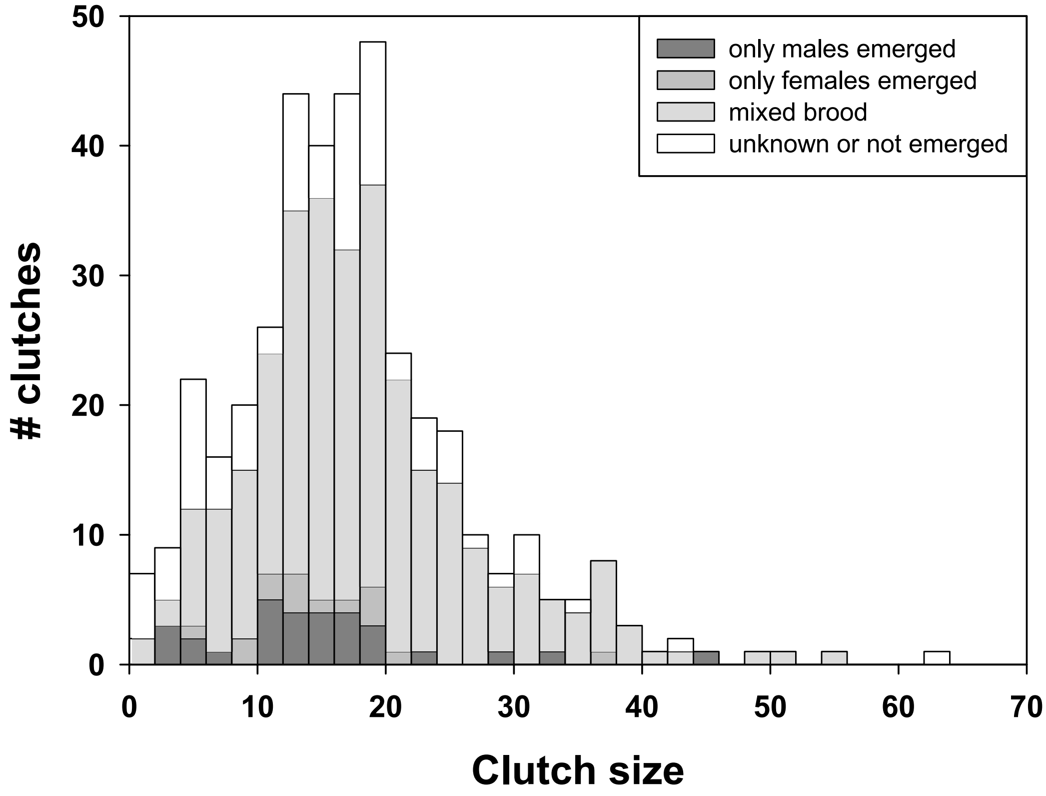


Figure 2.3 Natural clutch size distribution of *M. tristis* in *H. bicruris*.

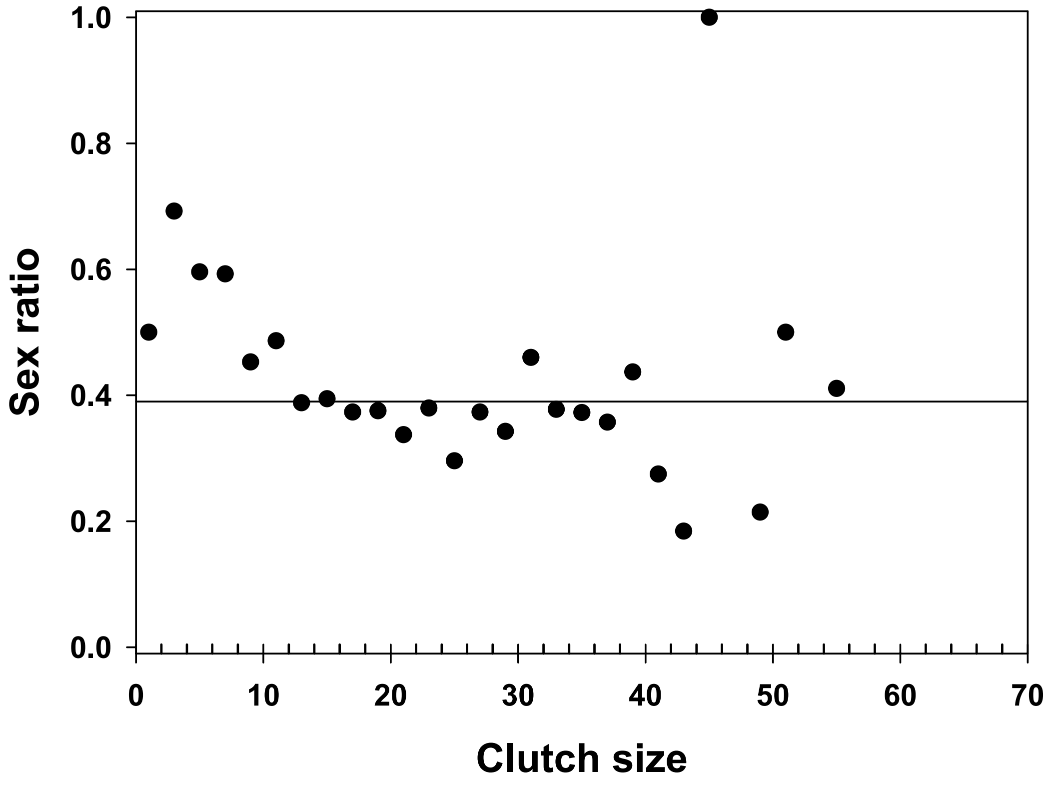


Figure 2.4 Average sex ratios (fraction of males) for different clutch sizes of *M. tristis* in *H. bicruris*. The horizontal line indicates the overall average sex ratio.

Hymenoptera

Braconidae

Microplitis tristis

The majority of parasitism was caused by *Microplitis tristis* Nees 1834 (Braconidae: Microgastrinae), a gregarious koinobiont endoparasitoid. On average 22.9 % of the collected *H. bicruris* caterpillars were parasitised by *M. tristis*. Larvae of *M. tristis* feed on the haemolymph of the caterpillars and, at maturity, they egress from the host and pupate next to it, usually in the soil, but occasionally in a secondary seed capsule. Because the life cycle of the parasitoid is much shorter than that of its host (Elzinga *et al.* 2002), we assume that several overlapping generations occur per year. *M. tristis* diapauses as prepupae in the cocoon if reared under autumn conditions.

On several ocassions the parasitoid was observed in the field parasitising L5 hosts that were exposed on a secondary seed capsule, either completely or only with their abdomen. This corresponds to the observation that most collected parasitised caterpillars were in L5 (23.8%, n=331), although a small percentage of L4 (12.2%, n=36) caterpillars were also parasitised. By contrast, none of the caterpillars in L3 were parasitised. The clutch size ranged from 1 to 63 with an average of 18.0 ± 0.47 s.e. (Fig. 2.3). Although there was a large variation in clutch size, there was no differencein clutch size between caterpillars collected as L4 and L5 (t-test, t =-1.287, d.f.=365, *P*=0.2).

The sex ratio of the clutches was female biased with a mean of 0.39, significantly deviating from 0.5 (Chi-square test, $\chi^2_{0.05,1}$ =233.9, n=4813, $P<0.001$), even though from approximately 10 % of the clutches only males emerged (Fig. 2.4). From approximately 5 % of the clutches only females emerged but survival of most of these single sex female clutches was much lower than 50 %. This is different from single sex male clutches where a majority had a survival higher than 50 %. The largest clutch producing only males was 45 with 36 males surviving to eclosion. A large proportion of males seem to be produced in clutches smaller than 10 (Fig. 3.4). Sex ratio was not influenced by the instar (L4 or L5) at which the host was found nor by clutch size (GLMs, all $P>0.05$).

Shenefelt (1973) lists many host records of the palearctic *M. tristis*, all Lepidoptera, including *Hadena* species, of which only a few, like *Aglais urticae,* occur in our study area besides *H. bicruris*. Our observations in the field and laboratory suggest that the parasitoid female is strongly attracted to the plant, damaged seed capsules and frass from *H. bicruris* and that newly emerged females in a small vial will easily attack *Hadena* *bicruris* caterpillars when offered. Caterpillars of other species (S*podoptera exigua, Mamestra brassicae, Chrysodeixis chalcites* and *Aglais urticae*) offered in a similar way did not initiate parasitism behaviour (n = approximately 5 per species) although successful larval development in *Chrysodeixis chalcites* was possible after parasitism was forced (by pressing the abdomen on the host). This was not observed in the other species.

Bracon variator

The most important ectoparasitoid present in the system is the gregarious *Bracon variator* Nees 1812 (Braconidae: Braconini). Of the caterpillars collected, 4.9 % were found either dead or paralysed and covered with cocoons, larvae or eggs of this parasitoid. Females inject paralysing venom into the host before oviposition on the outside of the host. The larvae then perforate the cuticle with their mandibles and imbibe nutrients from the paralysed or dead host, before spinning cocoons next to it. Hibernation occurs as prepupa in the cocoons if reared under autumn conditions. *B. variator* attacks hosts that are hiding in secondary, but occasionally also primary, seed capsules. In a few cases the paralysed caterpillar also contained larvae of *M. tristis*. Except in one case, the latter could not develop and died inside the host. On one occasion *B. variator* larvae were found developing on a host from which *M. tristis* larvae had already egressed. Host size ranged from L3 to L5 although the majority are parasitised as L5 (L3=2, L4=36, L5=127, unknown=42). On several occasions we observed *B. variator* attacking hosts by entering secondary seed capsules containing L4 and L5 hosts.

Although the average clutch size increased with instar attacked (2.5 for L3 (n=2), 5.09 for L4 (n=32) and 6.27 for L5 (n=118)), this difference was not statistically significant (ANOVA, $F_{2,151}$ =1.61, *P*=0.20). Average clutch size for all collected broods (including those from the experimental garden) was 5.96 ± 0.31 with most broods within the range of 1-6, but a maximum of 27 was observed (Fig 2.5).

The overall sex ratio was 0.34 and was significantly different from 0.5 (Chi-square test, $\chi^2_{0.05,\,1}$ =96.3, n=941, *P*<0.001, Fig. 2.6). The majority of the clutches are single sex especially if clutch size is smaller than six. The mean sex ratio did not change significantly with clutch size, for all clutches combined, or for mixed clutches only (GLMs, all *P*>0.05).

Many host species in different insect orders have been described for this palearctic parasitoid, such as Tephritidae, Syrphidae, Coleoptera and Lepidoptera (Pickard and Rabaud 1914, Freese 1995, Georgiev and Samuelian 1999, Diaconu and Lozan 2000) including *H. bicruris* (as *Harmodia capsincola*) (Shenefelt 1978). Most species appear to be living in confined spaces like flowerheads, leaf rolls or seed capsules.

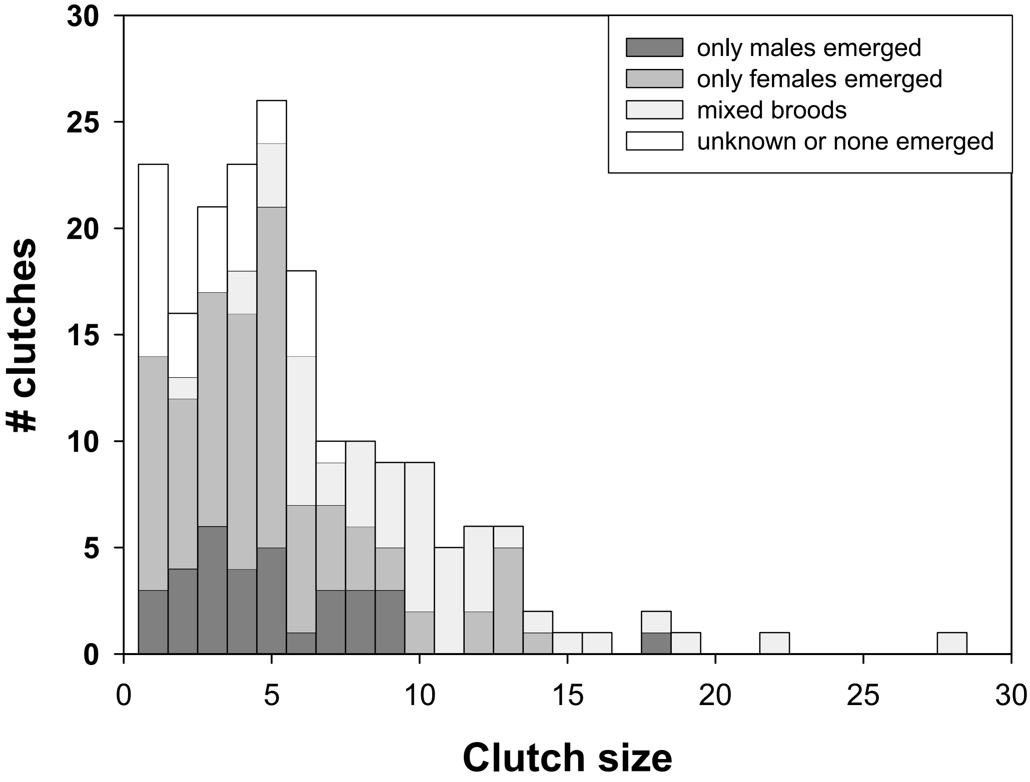


Figure 2.5 The natural clutch size distribution of *B. variator* on *H. bicruris.* Figure is based on data obtained in the field and the experimental garden.

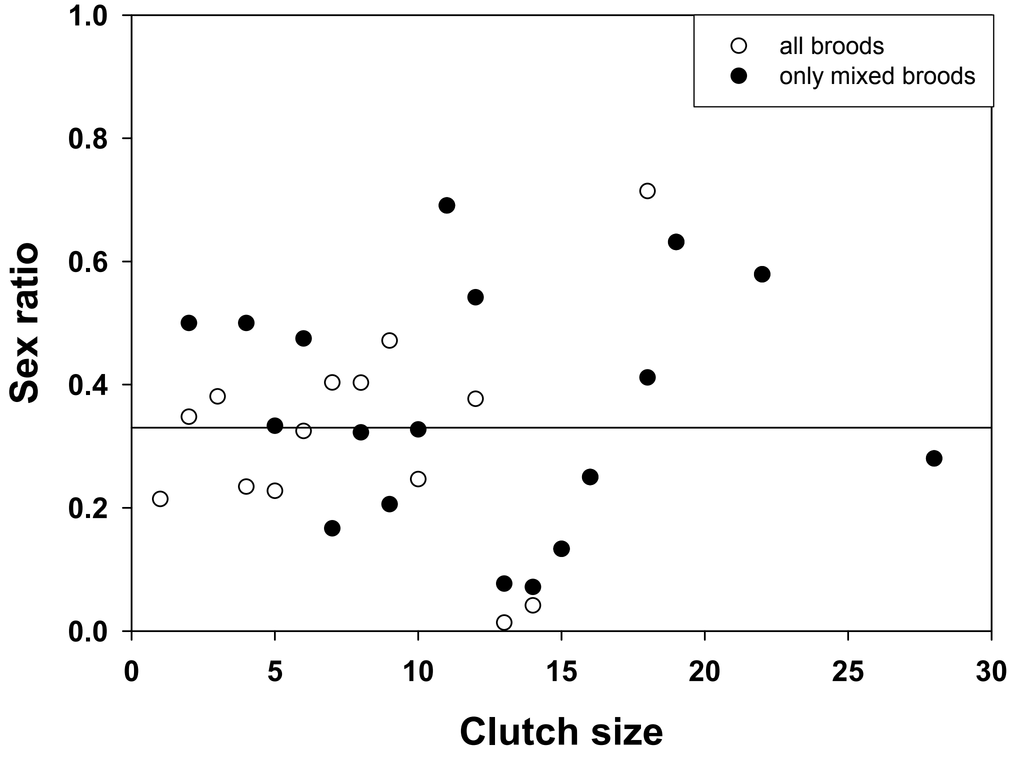


Figure 2.6 Average sex ratio (fraction of males) for different clutch sizes of *B. variator* in *H. bicruris*. The horizontal line indicates the overall average sex ratio. Figure is based on data obtained in the field and the experimental garden.

Bracon brevicornis

The gregarious ectoparasitoid *Bracon brevicornis* Wesmael 1838 (Braconidae: Bracononi) was found only once, in the experimental garden (1-7-2003). A paralysed L5 caterpillar hosted a clutch of 14 larvae that developed into nine males and four females within a few weeks. *B. brevicornis* is known from many lepidopteran hosts in Europe, Asia, Africa and introduced in America and is an important parasitoid of several pest species, but until now was not observed as a parasitoid of *H. bicruris* (Shenefelt 1978, Jackson and Butler 1984, Temarak 1984).

Ichneumonidae

Eurylabus tristis

Eurylabus tristis (Gravenhorst 1829) (Ichneumonidae: Ichneumoninae) is a solitary koinobiont larval-pupal endoparasitoid that was observed in 11.7 % of the collected caterpillars. The larva of this species remains as an L1 in the caterpillar and completes development only after the host has pupated. In some dead and dissected caterpillars, including a few that had been parasitised by *M. tristis*, larvae of *E. tristis* were observed, in one instance apparently fighting a second conspecific larva with its mandibles. Diapause occurs in the larval stage in the host pupa when reared under autumm circumstances. Occasionally, caterpillars that were collected early in the season and reared under summer conditions went into diapause after pupation. From some of these pupae *E. tristis* emerged after diapause. Adults were observed attacking L5 hosts that were hiding in secondary seed capsules by inserting their abdomen throught the entrance hole. Only 14 out of the 198 collected caterpillars parasitised by *E. tristis* were L4, whereas the rest were L5. The sex ratio was not different from 0.5 (Chi-square test, $\chi^2_{0.05,1} = 0.33$, n=194, n.s.).

Only four noctuid hosts are mentioned in Taxapad (Yu 1998) for this palearctic species: *H. bicruris*, *H. albimacula, H. perplexa* and an unlikely record on *Panolis flammea*, a pine feeding noctuid.

Ophion pteridis

Ophion pteridis Kriechbaumer 1897 (Ichneumonidae: Ophioninae) is a solitary koinobiont endoparasitoid, which had parasitised 3.4 % of the caterpillars. It feeds on host haemolymph but eventually consumes the whole soft tissue of the host before egresssion. Occasionally it egresses from the host just after pupation, but mostly from L5 hosts just before they pupate. It pupates next to the host in the soil in a brown cocoon with a distinct lightbrown band. *O. pteridis* was found only rarely in hosts collected as L4 (n=4) and most often in L5 (n=55). Facultative diapause occurs as prepupae in the cocoons. The sex ratio observed from 19 individuals was 0.26 and this was significantly different from 0.5 (Chi-square test, $\chi^2_{0.05,1}=6.37$, n=19, $P=0.04$).

In Taxapad (Yu 1998) only three noctuid hosts are mentioned: *Lacanobia* (= *Mamestra*) *pisi*, *L. oleracea* and *Callopistria juventina*. Brock (Brock 1982) also mentions *L. pisi* and *L. oleracea* and observes that it quite regularly parasitises night feeding noctuid caterpillars in seed pods of *Campanula*, but that must be a misidentification for *Silene*, as he refers to a few species of *Hadena*, including several observations on *H. bicruris,* that do not occur on *Campanula*. *O. pteridis* is a palearctic nocturnal parasitoid that can be trapped with light traps (Nabli *et al.* 1999), and is widely distributed and fairly common in the Netherlands (Oosterbroek 1978).

Erigorgus cerinops

Two females of *Erigorgus cerinops* (Gravenhorst 1829) (Ichneumonidae: Anomaloninae) were found in caterpillars collected as L5 (on 20-6-2003 and 29-8-2003 resp.). This larval-pupal endoparasitoid ecloses from the host pupa within the same season or alternatively after diapause. Several noctuid hosts are mentioned in the literature for this palearctic parasitoid; this is the first published record from a *Hadena* species (Schnee 1993, Yu 1998).

Scambus sp.

On nine occasions a *Scambus* species (Ichneumonidae: Pimplinae) was observed as a parasitoid of *H. bicruris*, mostly on L4 and occasionally on L5 hosts. Three species could be identified, and a few others did not develop into adults. These *Scambus* species are all solitary ectoparasitoids that paralyse their host before depositing eggs on the outside of the host. They spin cocoons next to the carcass, after feeding primarily on imbibed host haemolymph. The three *Scambus* species are all palearctic but *S. buolianae* has been released as a biocontrol agent in Canada for the European pine shoot moth *Rhyacionia buoliana* (Lepidoptera: Tortricidae) (Leius 1961).

Scambus brevicornis (Gravenhorst 1829), was collected five times (28-8-2001, 29-8-2001, 26-6-2002, 12-6-2003 (2x)) on paralysed or dead L4 and L5 hosts. One was found on a caterpillar from which *M. tristis* had already eclosed. All identified specimens were females. This species has been found in large numbers on caterpillars of the leafroller *Adoxophyes orona* in apple orchards in the Netherlands (Evenhuis and Vlug 1983) but occurs on insect hosts from many different orders like Coleoptera, Diptera (Tephritidae), Hymenoptera, and many Lepidoptera (Solbreck and Sillén-Tullberg 1986, Molau *et al.* 1989) including *Hadena rivularis* (Yu 1998). It appears that most of these hosts occupy flower heads, leaf rolls, seed capsules or other confined spaces.

Scambus buolinae (Hartig 1838) and *Scambus annulatus* (Kiss 1924) were found twice (on 11-9-2001 and 10-9-2002) and once (on 3-7-2003) resp., as larvae and they were all females. Like *S. brevicornis* these two species have a wide range of mostly confined living hosts (Leius 1961, Bauer 1986, Godfray *et al.* 1995, Jordan 1995, Yu 1998, Hellrigl and Ambrosi 2000, Kruess and Tscharntke 2000). *Hadena* had not yet been mentioned as a host.

Unidentified species

We were unable to identify a single *Hyposoter* (Ichneumonidae: Campopleginae) female to species level. It developed within the same season from a caterpillar collected on 20-6-2003 as L5.

Diptera

Tachinidae

Several tachinids did not emerge from their pupae for unknown reasons and could therefore not be identified at the species level. In total, tachinids contributed to only 0.6 % of the parasitism (Table 2.2). Most tachinid larvae egressed from L5 hosts, whereas on three occasions the larvae emerged after pupation of the caterpillar, of which two occurred after diapause of the host pupa. Four gregarious koinobiont endoparasitoid tachinid species were identified.

Blondelia nigripes (Fallén 1810) (Exoristinae: Blondeliini). This species was obtained from three hosts. One clutch contained three females, one was a solitary male, and the third clutch (found in an experimental plant patch near the river Waal), consisted of three females and one male. The parasitised caterpillars were collected as L5 (at 12-6-2003, 3-7-2003 and 7-7-2003 resp.) and the parasitoids emerged several weeks later in the laboratory. *Blondelia* species are equipped with a piercing ovipositor which is used to inject an incubated egg directly into the body of a host (Dowden 1933). *B. nigripes* is a very polyphagous species attacking numerous Lepidoptera from different groups, mostly unhairy caterpillars, and also occasionally Tenthredinidae and Symphyta (a.o. Tschorsnig and Herting 1994, Ford *et al.* 2000, Avci and Kara 2002).

A second species was *Siphona geniculata* (De Geer 1776) sensu Andersen (formerly *S. cristata* Fabricius 1805) (Tachininae: Siphonini). Three individuals eclosed from one caterpillar, collected as L5 (at 12-6-2003) from which two females emerged a few weeks later. This gregarious species has been associated mainly with large Lepidoptera, particularly Noctuidae (Ford and Shaw 1991, Belshaw 1993, Tschorsnig and Herting 1994, Ford *et al.* 2000)

Phryxe vulgaris (Fallén 1810) (Exoristinae: Eryciini). This species was recorded twice in *H. bicruris* larvae, one collected as L5 (on 12-6-2003), the other as L4 (on 29-8-2003) from which resp. three females and one male, and one female plus one male developed; the first clutch egressed from the caterpillar, the second from the host pupa after diapause. It has been found previously on *H. bicruris* (in Belshaw 1993) and is extremely polyphagous on different families of Lepidoptera with relatively large caterpillars (Tschorsnig and Herting 1994). It is also known from the nearctic region (O'Hara 1999).

Phryxe nemea (Meigen 1824) (Exoristinae: Eryciini). We identified this species from one caterpillar, collected as L5 (at 23-6-2003), from which five females developed after eclosion from the host larva. This species is highly polyphagous on exposed caterpillars of many families of Lepidoptera, including *H. bicruris* (Belshaw 1993), although extremely hairy caterpillars seem to be avoided (Tschorsnig and Herting 1994, Ford *et al.* 2000). *Phryxe* species deposit their eggs on the outside of the host. After hatching shortly after, the larvae will then burrow themselves into the host.

Nematodes

We observed parasitism by nematodes in two instar 5 caterpillars (13-9-2001 and 3-7-2003), of which the second had already been parasitised by *M. tristis*. We presume that these large (up to 18 cm long) nematodes were of the genus *Hexamermis* (Nematoda: Mermithidae) (Wouts 1984), but they could not be identified further.

Hyperparasitoids

We found six species of hymenopteran hyperparasitoids associated with the primary parasitoids *M. tristis* and *B. variator*. Only one species is a true hyperparasitoid (on *M.*

tristis), the others are all pseudohyperparasitoids attacking cocoons or external larvae. Nine out of the 33 clutches of *M. tristis* that we collected in the field and in the experimental garden as recently eclosed larvae or as cocoons contained a hyperparasitoid and 21 out of 73 cocoon clutches of *B. variator* (plus two larval clutches).

Ichneumonidae

Mesochorus lanceolatus

Mesochorus lanceolatus Schwenke 1999 (Ichneumonidae: Mesochorinae) is a true hyperparasitoid that parasitises *M. tristis* larvae when they are still in the host. In total we found six clutches of *M. tristis* parasitised by *M. lanceolatus*. Only three were recorded from the field, whereas the others were recovered from experimental plant plots along the river Waal and in the experimental garden. In all cases the clutches of *M. tristis,* ranging from 14 to 30 individuals, were only partly parasitised so that in addition to broods of 3 to 28 hyperparasitoids, adult *M. tristis* also eclosed. One of the broods of *M. lanceolatus* contained only males which might be a sign of oviposition by an unmated female. The other broods contained both females and males. All broods were collected in July or September 2000, 2001 or 2002. Half of the broods diapaused within the cocoons of *M. tristis*.

Host use by many of the 314 species of *Mesochorus* described for Europe is largely unknown, but all species appear to parasitise Braconidae, Ichneumonidae and Tachinidae (Schwenke 1999). Often, they seem to be specialised on a primary host species and not on the secondary host, although some hyperparasitise many Lepidoptera species. Only one specimen from Sweden has been described and no previous host records exist (Schwenke 1999) for *M. lanceolatus*.

Gelis sp.

We found two species of the idiobiont genus *Gelis* (Ichneumonidae: Cryptinae) developing solitarily on cocoons of *M. tristis* and *B. variator*.

Gelis agilis (Fabricius 1775) was found on cocoon clutches of both *M. tristis* and *B. variator* (on 8-7-2002 and 29-8-2001 resp.). We found three small broods in the natural plant populations; three were obtained from a clutch of 16 and four from a clutch of four *M. tristis* cocoons and two from a *B. variator* clutch of four. Also Schwarz and Shaw (1999) observed that *G. agilis* was found in small broods, usually parasitising only a small fraction of the clutch of the primary host. We were also able to rear this species on

Cotesia glomerata cocoons in the laboratory for a few generations. Unmated females produced small numbers of female offspring, confirming that this species is thelytokous (Schwarz and Shaw 1999). We did not observe any hibernation. They can be extremely long-lived, > 2 months at 25 ºC under laboratory conditions (J.A. Harvey, pers. obs.), and probably overwinter as adults (Schwarz and Shaw 1999). *G. agilis* has been found on many hosts, mainly small Lepidoptera, such as Coleophoridae (presumably as a primary parasitoid) and many Hymenoptera (Lei *et al.* 1997), including a *Bracon* sp. on *H. bicruris* (Schwarz and Shaw 1999)

Gelis hortensis (Christ 1791) was found twice in *M. tristis* and once in *B. variator* cocoons from clutches that had been partly parasitised. One parasitised *M. tristis* clutch (15 from a clutch of 30) was found in a natural plant population (at 26-6-2002) and the other (six from nine) and the one on *B. variator* (one from one), in the experimental garden (September 2002 and August 2003 resp.). Broods consisted of both apterous males and females. Two females (the rest of the brood had emerged before collection) and one male from the brood in *B. variator* hibernated, which is in contrast to the findings for this species by Schwarz and Shaw (1999), who did not observe any pre-adult hibernation. We were able to rear the species on *Cotesia glomerata* cocoons in the laboratory for one generation. Parasitism rates of these *Cotesia glomerata* cocoons were very small and only males emerged, indicating that the females might not have mated succesfully. The species has been found parasitising Lepidoptera, mostly species of Coleophoridae (presumably as primary parasitoid) and many Hymenoptera, especially Braconidae (as pseudohyperparasitoid) (Schwarz and Shaw 1999).

Chalcidoidea

All other pseudohyperparasitoids that were found developing on *M. tristis* and *B. variator* cocoons belong to the family Chalcidoidea. The three identified Chalcidoidea are palearctic species (Noyes 2001) and were mentioned in a recent species list for the Netherlands (Gijswijt 2003).

Baryscapus endemus (Walker 1839) (Eulophidae: Tetrastychinae) was the most abundant pseudohyperparasitoid. In the field it was found twice, once on a clutch of *M. tristis* (12-9-2001) and once on *B. variator* (14-9-2001). In the garden we observed it twice on *M. tristis* and 12 times on *B. variator* (collected in July, August or September 2002 and 2003). Although most hosts were collected as cocoons, twice hosts were collected as larvae (*B. variator* and recently emerged *M. tristis*), suggesting that it is an endoparasitoid. Broods ranged from one to five individuals, often parasitising host clutches only partly. All collected specimens (n=35) appeared to be females, suggesting thelytokous reproduction. Almost all broods hibernated (one only partly). This species has been mentioned for various hosts in different insect orders (Coleoptera, Hemiptera, Hymenoptera and small Lepidoptera) and as a hyperparasitoid on hymenopteran parasitoid species including several braconids (Noyes 2001).

Pteromalus vibulenus (Walker 1839) (Pteromalidae: Pteromalinae) was found once in the field on cocoons of a clutch of *M. tristis* (28-8-2001) and four times in the garden on cocoons of *B. variator* (August and September 2001 and 2003). Once, two individuals emerged from one host cocoon, showing facultative gregariousness. Broods ranging from one to four consisted of males, or were of mixed sex. Hibernation occurs when kept under autumn conditions. *P. vibulenus* has been found on parasitoid species and many species of primary hosts (orders: Coleoptera, small Lepidoptera, Hymenoptera) and is cited several times as hyperparasitoid of *Apantales glomaratus* on *Pieris brassicae* (Noyes 2001).

Pteromalus chrysos Walker 1836 (Pteromalidae: Pteromalinae) was observed once in the field developing in cocoons of *B. variator* (1-8-2002). The clutch of five hosts was partly parasitised (brood of four) and emerged without hibernation. It has been identified previously from tachinid and hymenopteran parasitoids including several *Apanteles* species and other Braconids and from many primary hosts (Coleoptera, Diptera, Hymenoptera and small Lepidoptera) (Noyes 2001).

Discussion

The parasitoid complex associated with H. bicruris

Figure 2.7 gives an overview of the parasitoid complex associated with *H. bicruris* as observed in our study. On average 44 % of all collected *H. bicruris* caterpillars were parasitised, but because collecting the caterpillars could have reduced the time they were vulnerable to parasitism, natural parasitism rate might even be higher. Four species of parasitoids, *M. tristis, E. tristis, B. variator* and *O. pteridis* are by far the main species of parasitoids of *H. bicruris*. The other species of parasitoids were observed only once or a few times and not always in every year.

The pattern observed is not very different from that described for many other species of Lepidoptera, although the number of parasitoid species is quite high. In Lepidoptera the average number of parasitoid species as extracted from global literature is 9.4 (Price 1994), but the average number of parasitoid species in Lepidoptera that leave their refuge during development ("mixed" species sensu Hawkins (1993)) is only 5.1. However, it is unlikely that all studies on which these numbers are based, were using as comprehensive data sets as presented in this study. Therefore parasitoid species that are rarely encountered on a host might have been missed in these studies.

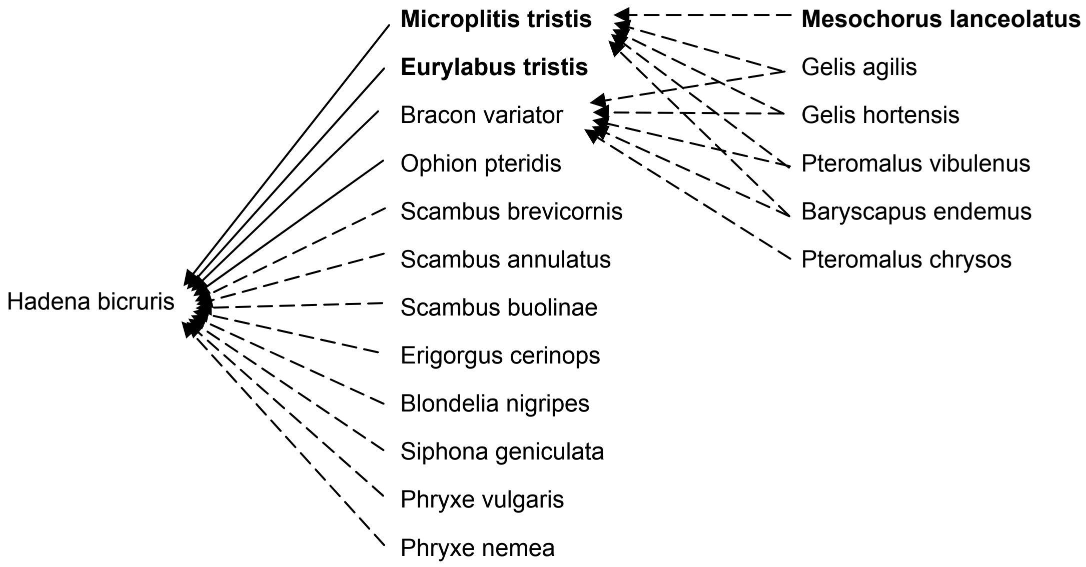


Figure 2.7 The parasitoid complex as observed on *H. bicruris* in the Netherlands along the river Waal. Solid lines show the most important parasitoid species. Bold names indicate possible specialist species.

Several sources list additional parasitoid species associated with *H. bicruris*, although the reliability of these records may be doubtful. Shenefelt (1980) lists several other Braconids; *Apanteles sericeus*, *Microplitis spectabilis* and from *Hadena sp. Microplitis mediator*. In the Taxapad database (Yu 1998) we find *Agrypon flexorium* (Anomaloninae), *Cotesia praepotens*, *Enicospilus ramidulus* (Ophioninae), *Meringopus turanus* (Cryptinae), *Netelia vinulae* (Tryphonina), *Ophion luteus* and *O. scuttelaris* mentioned as parasitoids of *H. bicruris*. In addition, several species are listed that attack other *Hadena* species. Other tachinids that have been mentioned as parasitoids are *Epicampocera succincta* (Exoristiniae: Eryciini) (Ford and Shaw 1991) and *Compsilura concinnata* (Exoristinae: Blondeliini) (Hammond & Smith, 1953 in Belshaw 1993). All these records do not come from Dutch source populations and might suggest regional differences in the composition of parasitoid complexes. As *H. bicruris* is widespread in

Europe it would be necessary to study the parasitoid complexes in other countries to provide more information about these possible regional differences. Because many other *Hadena* species have similar lifestyles (i.e. feeding on seed capsules of Caryophylaceae) it would be interesting to compare their parasitoid complexes as well.

For several Lepidoptera it has been observed that egg- or egg-larval parasitoids are an important mortality factor (Hirose 1994). Although we collected *H. bicruris* eggs on several occasions, we have never observed any parasitoids developing from eggs or from the emerging larvae. Although the eggs of *H. bicruris* are usually found on the ovary deep in the calyx of the flower, this does not necessarily protect them from egg parasitoids. For the related *H. compta* on the plant *Dianthus sylvestris*, it has been observed that the parasitoid *Exetastes rufipes* (Ichneumonidae: Banchinae*)* uses its prolonged ovipositor to reach the egg or newly hatched caterpillar (Erhardt 1989). Eggs of *H. bicruris* should be collected more frequently to observe whether any parasitoid attacks them in the field. Furthermore, as we never collected pupae of *H. bicuris* from the soil, we do not know whether pupal parasitism occurs.

Level of specialisation of the primary parasitoids on H. bicruris

Determining the level of specialisation of a parasitoid on a specific host is extremely difficult. It is impossible to test whether all species occuring in a region are potential hosts for a parasitoid. Therefore one often has to rely on literature describing observations of a parasitoid developing on a certain host species. However, there are many objections and problems to abstracting host ranges from other literature (Shaw 1994). Among the main objections are the numerous mis-identifications and the uncertain status of names of both host and parasitoid species, the biased sampling efforts towards pest and easy to sample host species, and the equal weight given to regular and “freak” associations (Shaw 1994). For example, a closer study on *Cotesia* (*=Apanteles) glomerata*, a braconid related to *M. tristis*, revealed that 49 out of 57 mentioned host species appeared to be wrong (Shaw 1982).

However, some general observations can be made. Koinobiont endoparasitoids (with the exception of tachinids, see (Belshaw 1994)) are generally seen as relatively specialist parasitoids (Shaw 1994). Due to their intimate contact with the host tissue, which requires in most cases adaptation to the host immune defence system and regulation of host growth and behaviour, it is generally believed that coevolution between hosts and parasitoids has been very important for these species (Whitfield 1994).

However, not only physiological constraints are believed to limit current host range, also host searching behaviour is often highly adapted to a specific host species or group (Askew 1994).

Recent studies have investigated the genetic structure of a parasitoid on different host species. It appeared that *Cotesia* species on Melitaeini actually consist of genetically different clades specialised on different host species, suggesting that host specialisation might even be more common than originally thought, especially for koinobiont endoparasitoids (Kankare and Shaw 2004).

Many parasitoids (but especially idiobiont ectoparasitoids) that are considered as generalists and thus capable of developing on many different insect species or even orders are actually specialised on a certain habitat or an aspect of host behaviour, such as living concealed in a plant structure (Shaw 1994).

Most of the parasitoid species observed on *H. bicruris* in this study might be considered as generalists. Especially those that were encountered only rarely like the *Scambus* species, the tachinids and others are highly polyphagous on different insect taxa. The *Scambus* species, but also the much more abundant *B. variator*, seem to be specialised on hosts that live concealed in plant tissues (Shaw 1994), which can also be inferred from the relatively long ovipositor these species possess. *O. pteridis* is observed on several noctuid species but is regularly found on *H. bicruris* (Brock 1982) and might be considered as an oligophage, specialised on a limited number of noctuids.

Only two species might be considered as specialists in the region under study; *M. tristis* and *E. tristis.* Although Shenefelt (Shenefelt 1973) lists several other hosts for *M. tristis,* these host species probably do not occur within the regionally realised host range, either because they are very unlikely to occur in the study area or because they are from a completely different family of Lepidoptera (e.g. *Aglais urticae*) that may rely on an erroneous original record. Although *M. tristis* can apparently develop in some noctuid species like *Chrysodeixis chalcites*, this species does not elicit oviposition behaviour and it oviposits only when forced. By contrast, *M. tristis* showed a clear response to odours of *S. latifolia*, its (damaged) seed capsules, the frass of larval *H. bicruris* and the caterpillar itself, suggesting a strong adaptation to this host. Although only a rigorous rearing of alternative hosts can reveal whether *M. tristis* is a true specialist, we think that this parasitoid is, at least regionally, a specialist parasitoid. Also *E. tristis* is very likely to be a specialist on *Hadena* locally. Although it has been recorded from a different host as well (Yu 1998), this record is unlikely and furthermore, that host is not present in the study area.

Summarising, we think that *H. bicruris* is the only host in the region for *Microplitis tristis* and *Eurylabus tristis*, and it is an important host for *Ophion pteridis* and *Bracon variator*. All other parasitoids will probably have different alternative hosts in the region that are much more important for their population numbers.

Hyperparasitoids

In some multi-trophic systems hyperparasitism has a major impact on the population dynamics of the primary parasitoid (Lei *et al.* 1997, van Nouhuys and Hanski 2000). In our system around a quarter of the clutches of *M. tristis* and *B. variator* seem to be hyper-parasitised. We did not observe one particular species responsible for most hyperparasitisms of *M. tristis* but the most abundant hyperparasitoid in the garden on *B. variator* was *B. endemus*. Because *B. variator* always pupates in seed capsules, it is probably much more vulnerable than *M. tristis* which usually pupates in the soil.

All collected hyperparasitoids are highly generalistic, with the exception of *M. lanceolatus*. Although host ranges of *Mesochorus* species are not well documented it has been suggested that most of them depend quite heavily on the secondary host, i.e. in host searching/acceptance they home in on the Lepidopteran host, rather than on their tachinid or hymenopteran primary hosts (Schwenke 1999). The host ranges of the *Gelis* species and the Chalcidoidae are very wide and they can potentially attack most parasitoid species that produce small cocoons or pupae. It is even possible that they attack several primary hosts but, as Shaw (Shaw 1994) already mentioned, in much of the literature the distinction between primary and secondary (hyper)parasitism has not always been made correctly. In conclusion, we think that there are no large effects of hyperparasitoids on the population dynamics of the primary parasitoids associated with *H. bicruris* (except maybe for *B. variator*) and vice-versa, but further study is needed to support this thought.

Effects of the parasitoids on the plant-host interaction

The moth *H. bicruris* is generally considered to be a parasite of its host plant, *S. latifolia* as the effects of pollination by the adult moth do not outweigh the detrimental effects through seed predation (Brantjes 1976b, Bopp 2003). Several studies mention the possibility that due to the high rate of parasitism the number of seeds destroyed by the herbivore hosts might decrease (Biere *et al.* 2002, Bopp 2003, Elzinga *et al.* 2003) and even to such an extent that the positive effect of pollination by the adult *H. bicruris* might counteract the negative effects of herbivory by the moths offspring. This would lead to a mutualism or commensalism, in which the plant and the moth both benefit from each other. However, our data on parasitism do not support this idea.

First, only a small percentage of parasitism is caused by idiobionts (*B. variator*, *Scambus* sp.) which arrest host growth. The other parasitoids are koinobionts and allow the host to continue to feed and grow for variable periods after parasitism. Although for *M. tristis* it has been experimentally observed that caterpillars feed less when parasitised, this has only been shown for clutches much smaller than the natural clutch sizes (Elzinga *et al.* 2003). *E. tristis* may have little or no influence at all on host feeding as the larva develops only after pupation of the caterpillar. *O. pteridis* also will not cause a substantial decrease in the number of destroyed seeds as it emerges just prior to pupation (or even just after).

Secondly, even if parasitoids reduce the food consumption by *H. bicruris*, most parasitism occurs in the latest stages of the caterpillar when one or more seed capsules have already been destroyed. From this study it is clear that most parasitism occurs in L4 or L5 hosts and that only a very limited number of hosts are attacked as L3 or earlier. Only *B. variator* seems to access hosts occasionally through the small entrance hole of a primary seed capsule, that is often blocked by faeces, as they were observed to have parasitised L3 twice. Ovipositions by *B. variator* and *E. tristis* were observed on L4 and L5 caterpillars that were seeking refuge in empty secondary seed capsules. These have a large entrance opening through which *E. tristis* can insert its abdomen or allow *B. variator* to enter completely. When feeding from secondary seed capsules the abdomen of *H. bicruris* is often exposed while the head is concealed in the seed capsule. During this period the caterpillar is probably the most vulnerable for the tachinids that need to be in close contact to the host to oviposit their eggs and for parasitoids with short ovipositors, like *M. tristis*. All observations of ovipositions of *M. tristis* in the field were made on exposed L5 larvae. This is well in accordance with a performance experiment in which large caterpilars were found to be optimal for development of *M. tristis* clutches (Elzinga *et al.* 2003)

We conclude that, even in the presence of a high parasitism rate, the relationship between *S. latifolia* and *H. bicruris* is not to be considered mutualistic because the parasitoids do not substantially decrease the food consumption of *H. bicruris*. Whether the parasitoids have a positive impact on the plants through a decrease in the number of adult moths in the next generation, will depend on whether parasitism results in a decrease in the number of deposited moth eggs per plant.

Clutch size and sex ratio of the main parasitoids of H. bicruris

In *M. tristis* and *B. variator* clutch sizes vary considerably and are independent of host size. *M. tristis* produces an average clutch size of 18.0. This is very close to the optimum clutch size for *M. tristis* in large *H. bicruris* hosts under laboratory conditions, as has been shown in a previous experiment, where individual offspring weight and survival were compared for different clutch sizes (Elzinga *et al.* 2003). However, there is considerable variation in clutch size which is poorly understood. Experiments have shown that host size did not influence clutch size (Elzinga *et al.* 2003) and that parasitoid age could only cause a reduction in clutch size if the parasitoid reaches an unnatural high age or number of parasitisms (Chapter 6).

One of the reasons for smaller than optimal clutch sizes might be the that a parasitoid is unable to complete oviposition because the host, in defending itself, disturbs the female (Ikawa and Suzuki 1982). On the other hand, larger clutch sizes might be caused by superparasitism either by the same female or by a conspecific (Gu *et al.* 2003). In experimental rearings under favourable conditions, we have never observed clutches larger than 28 (Elzinga, pers. obs.), suggesting that the large clutches found in the field are caused by superparasitism.

B. variator produces an average clutch size of six, but again, a large variation was observed. However, *B. variator* is capable of varying its offspring size impressively. When clutch size is large, small parasitoids emerge, whereas small clutches can produce individuals that are much bigger (Elzinga, pers. obs.). The consequences for individual fitness of size differences are not known, but many studies of other parasitoid species show that small individuals live shorter and produce fewer eggs (e.g. Visser 1994). Like for *M. tristis*, the large clutches might be the result of occasional superparasitism.

Different species are expected to have different offspring sex-ratios depending on their mating strategy. In general, species that often mate with their siblings (Local Mate Competition) are expected to show a female biased sex-ratio (Hamilton 1967, Godfray 1994). In contrast, species that mate randomly are more frequently expected to produce an equal sex-ratio (Fisher 1930, Hardy 1992). In general, gregarious species are thought to have a higher degree of sibling mating than solitary species due to a high chance of mating within a clutch and indeed female-biased sex ratios are very common in gregarious hymenopterans or solitary species attacking gregariously living hosts (Godfray 1994). Our data do not show otherwise; *Eurylabus tristis*, a solitary parasitoid shows an almost perfect equal number of male and female offspring, in contrast to the gregarious *B. variator* and *M. tristis* who clearly show a female biased sex ratio.

However, there was a clear difference in the distribution of sex ratios across individual clutches between these two gregarious parasitoids. For *M. tristis* most clutches consist of mixed broods from which both males and females emerge. The larger number of single sex male clutches can be explained by the fact that a small percentage of females is unmated and produces unfertilised eggs that develop into males. *B. variator* on the other hand produces many more single sex clutches, including many female clutches. Not only are small clutches of up to six individuals almost all single sex, but also most of the larger clutches. Laying single-sex clutches may incur different types of benefit to a female parasitoid (Hardy 1992). First, it is possible that survival and development of siblings is greater in single-sex broods than in mixed broods. However our data on clutch survival do not indicate that mixed broods do less well. Therefore a second explanation might be more likely. In two close relatives to *B. variator*, *B. hebetor* and *B. serinopae*, but also in other Hymenopteran species it is shown that sex is determined by the segregation of several alleles at a single sex determination locus (Godfray 1994, Antolin *et al.* 2003). In species where sex determination is linked to heterozygosity at sex loci (so called complementary sex determination), outbreeding may reduce the probability of the production of unfertile diploid males (Antolin *et al.* 2003). Although we do not know whether *B. variator* possesses a similar sex determination system, the many single sex clutches give a strong suggestion that inbreeding is strongly avoided. The fact that several large clutches are mixed, may further suggest that these clutches are a product of superparasitism.

Conclusions

At least 14 species of both hymenopteran and tachinid species of parasitoids parasitise almost half of the caterpillars of *H. bicruris*, although 95% of the parasitism is caused by only four hymenopteran species. Although it is difficult to show specialisation on a certain host for a parasitoid, we think that the two main species of parasitoids, *M. tristis* and *E. tristis* are specialists. The other species will probably have alternative hosts in the region.

We found 6 species of mainly generalist hyperparasitoids on *M. tristis* and *B. variator*. Only *Mesochorus lanceolatus*, a true hyperparasitoid, might be a specialist on

M. tristis. Hyperparasitism probably does not have a strong impact on the populations of the primary parasitoids.

Three of the main parasitoids are koinobionts that attack the caterpillars at a late stage and will have only limited effect on seed herbivory. It is therefore unlikely that parasitoids will shift the parasitic relationship of the pollinating *H. bicruris* with *S. latifolia* to a more mutualistic one.

Both *B. variator* and *M. tristis* show a large variation in clutch sizes that cannot be explained by host size. Sex ratio in the gregarious species *M. tristis* and *B. variator* is female biased, in contrast to the solitary *E. tristis* which has a sex ratio of 0.5. *B. variator* produces many single sex clutches, suggesting that they strongly avoid inbreeding.

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Chapter 3

The effects of host weight at parasitism on fitness correlates of the gregarious koinobiont parasitoid *Microplitis tristis* and consequences for food consumption by its host, *Hadena bicruris*

with Jeffrey A. Harvey and Arjen Biere

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Abstract

Gregarious koinobiont parasitoids attacking a range of host sizes have evolved several mechanisms to adapt to variable host resources, including the regulation of host growth, flexibility in larval development rate, and adjustment of clutch size. We investigated whether the first two mechanisms are involved in responses of the specialist gregarious parasitoid *Microplitis tristis* Nees (Hymenoptera: Braconidae) to differences in larval weight and parasitoid load of its host *Hadena bicruris* Hufn. (Lepidoptera: Noctuidae). In addition, we examined the effects of parasitism on food consumption by the host. Parasitoids were offered caterpillars of different weight from all five instars and parasitoid fitness correlates, including survival, development time and cocoon weight, were recorded. Furthermore, several host growth parameters and food consumption of parasitised and unparasitised hosts were measured .

Our results show that *M. tristis* responds to different host weights by regulating host growth and by adjusting larval development rate. In hosts with small weights, development time was increased, but the increase was insufficient to prevent a reduction in cocoon weight and as a result parasitoids experienced a lower chance of successful eclosion. Cocoon weight was negatively affected by parasitoid load, even though host growth was positively affected by parasitoid load, especially in hosts with small weights. Later instars are more optimal for growth and development of *M. tristis* than early instars, which might reflect an adaptation to the life-history of the host, whose early instars are usually concealed and inaccessible for parasitism on its food plant, *Silene latifolia* Krause.

Parasitism by *M. tristis* greatly reduced total host food consumption for all instar stages. Whether plants can benefit directly from the attraction of gregarious koinobiont parasitoids of their herbivores is subject of current debate. Our results indicate that, in this system, the attraction of a gregarious koinobiont parasitoid can directly benefit the plant by reducing the number of seeds destroyed by the herbivore.

Introduction

Insect parasitoids are parasitic only during their larval stages. The free-living adults parasitise the host and the parasitoid larvae feed externally or internally on the host, which is eventually destroyed (Godfray 1994). Parasitoid fitness is ultimately dependent on host quality, which describes a range of host characteristics that affect parasitoid growth, development, and survival. Because parasitoids develop upon a finite resource represented by a single host individual, host quality may vary with such factors as host age, stage or size parasitised, and nutritional status during the course of parasitoid development (Sequeira and Mackauer 1992, Pettit and Wietlisbach 1993, Croft and Copland 1995).

Foraging parasitoid females may encounter hosts of different sizes during their reproductive life span and several strategies have evolved to ensure that females select only hosts that will have a sufficient nutritional quality available to their progeny. Some parasitoids, called *idiobionts* (Askew and Shaw 1986) attack non-growing stages of the host e.g. egg, pupa or adult stages or else permanently paralyse the host before oviposition. These hosts effectively represent a ‘fixed’ resource (but see Rivers and Denlinger 1995).

Alternatively, *koinobiont* parasitoids (Askew and Shaw 1986) attack hosts that continue feeding and growing for variable periods of time after parasitism. Hosts for koinobionts represent a dynamic resource that may vary by many factors in size between oviposition and host death (Mackauer and Sequeira 1993).

Many parasitoids are able to assess host size and will only parasitise hosts of a certain size and reject smaller or larger hosts during the host-selection process (van Alphen and Drijver 1982, Strand and Vinson 1983). Because encounter rates with hosts with an optimal size may be quite low during a parasitoid lifetime (Harvey *et al.* 2001), parasitoids that are able to exploit a range of host sizes may have a stronger competitive ability compared to more selective parasitoids (Slansky 1986). Parasitism of hosts across a broad size-continuum is therefore a common phenomenon amongst koinobionts, and available resources may therefore vary between parasitoid larvae by many factors during early development.

When attacking small, suboptimally-sized hosts, endoparasitoid larvae may suspend their development, and remain as a first instar until the host reaches an appropriate size or nutritional value (Harvey *et al.* 1994). On the other hand, the development of large hosts may be arrested because of factors injected into the host during the oviposition sequence

(Vinson and Iwantsch 1980, Fleming 1992, Shelby and Webb 1997). Both strategies reduce the selection for a fixed maternal response at oviposition by allowing parasitoids to exploit a wide range of host sizes or stages.

In many parasitoids, adult body size is positively correlated with reproductive success, with large females living longer, producing more eggs, and exploiting hosts more effectively than smaller conspecifics (Visser 1994, Harvey *et al.* 2001). For gregarious parasitoids, where more than one individual develops in or on a single host, variation in clutch size may affect competition between sibling larvae. In some parasitoid species (mostly idiobionts), female wasps are able to estimate host size and to adjust clutch size in accordance with available resources (Schmidt and Smith 1986). However, clutch size may also be influenced by the physiological condition of the foraging female parasitoid, which can be affected by such factors as host availability, adult size and age, previous experience, or egg load. As a consequence, the number of eggs laid on a certain host size may vary considerably and lead to differences in offspring fitness (Rosenheim and Rosen 1991, Vet *et al.* 1994).

Few studies have examined in detail optimal host usage strategies by gregarious koinobionts attacking a wide range of host sizes or stages (but see Harvey 2000). In situations where gregarious parasitoids are able to stabilise host-size related variation in food supply for the parasitoid larvae by regulating host growth or delaying development, clutch size decisions may thus be expected to vary little with host size at oviposition. In fact, only a few studies have reported clutch size adjustment for gregarious koinobionts (Sato *et al.* 1986, Schopf and Steinberger 1996, Harvey 2000), but many are known to be able to manipulate host growth in accordance with parasitoid load, such that more heavily parasitised hosts consume more plant tissues, and grow larger, than lightly parasitised or healthy hosts (Dushay and Beckage 1993, Alleyne and Beckage 1997, Harvey 2000).

Many solitary koinobiont parasitoids are known to regulate host growth by reducing total food consumption, with parasitised hosts attaining only a fraction of the size of unparasitised hosts (Slansky 1978, Huebner and Chiang 1982, Rohlfs and Mack 1983, Schopf and Steinberger 1996). Alternatively, gregarious species often increase host food consumption, leading to hosts that attain greater maximum terminal weights than healthy host larvae (Rahman 1970, Führer and Keja 1976, Beach and Todd 1986, Harvey 2000). Therefore, for a plant there is not always a direct benefit from parasitism of the herbivores (van der Meijden and Klinkhamer 2000). Larvae of the species studied here, *Hadena bicruris*, feed on the developing seeds of *Silene latifolia*, and we expect that parasitism by the gregarious koinobiont parasitoid *Microplitis tristis* will result in increased food consumption by its host, which might have direct consequences for the fitness of the plant.

In this paper we examine the developmental responses of the gregarious koinobiont endoparasitoid *M. tristis* to different weights of its larval host, the noctuid *H. bicruris*, and the consequences of host weight at parasitism and parasitoid load for parasitoid fitness correlates. Furthermore, food consumption patterns are compared between parasitised hosts with different weights and parasitoid loads and unparasitised hosts.

Material and methods

Experimental system

Hadena bicruris oviposits on the ovary of female flowers of *Silene latifolia* and a few other closely related caryophyllaceous plant species (Wirooks and Plassmann 1999). Directly after hatching the first instar larva (L1) chews a small hole in the ovary, enters the young fruit, and starts to feed on the ovules and developing seeds. It will stay in the seed capsule until it is has consumed all the seeds, which usually occurs when the larva has reached its fourth or fifth (hereafter L4 and L5, respectively) host instar. At this stage the larva leaves the seed capsule and starts feeding on other seed 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 (Biere *et al.* 2002). At maturity, the larva leaves the food plant and pupates in the soil.

Microplitis tristis is a gregarious koinobiont endoparasitoid that is a specialist on larvae of *H. bicruris* (Shenefelt 1973). During development, larvae of *M. tristis* feed primarily on host haemolymph. When larval growth is complete, mature parasitoid larvae egress from the host by chewing a hole through the host's cuticle. The parasitoid larva then spins a cocoon next to the host and pupates. The host remains alive for several days, but eventually perishes without completing development. Host and parasitoid can be found from May until September in populations of *S. latifolia* in the Netherlands, and at least two overlapping generations have been recorded.

Rearing

Host eggs were obtained from adults collected as larvae from several locations in the province of Gelderland, The Netherlands. Hosts were reared individually on artificial diet (Elzinga *et al.* 2002) that was refreshed every 4 days, at 20 ºC, with L16/D8 photoperiod and 70 % r.h.

The culture of *M. tristis* originated from adults emerged from parasitised larvae of *H. bicruris* collected from several locations in the province of Gelderland, The Netherlands. Rearing was as described by Elzinga *et al.* (2002). To retain the cultures over longer periods, adult wasps were stored for several weeks at 4 ºC in the dark. Parasitoids were fed with undiluted honey and were mated by placing approximately 15 females and males together in 12 cm diameter Petri dishes for up to 48 h. After mating, the females were stored individually in separate vials (30 ml Coulter Counter cups) again a few days before being given access to hosts.

Experiments

Host instars of *H. bicruris* were determined on the basis of head capsule widths, which do not overlap between instars (Elzinga, unpubl. data). From each host instar, L1-L5, larvae with different weights were selected, avoiding hosts just moulted or ready to moult. Before parasitism, caterpillars were dipped into a solution containing mashed *S. latifolia* seed capsules to stimulate parasitism behaviour by the parasitoid. A caterpillar was offered individually to an individual female parasitoid inside a vial with a brush or a pair of tweezers. For each instar group of larvae we used 15 naive female parasitoids of similar age. Due to this limited number of parasitoids, females had to parasitise more than one host (on average 2.7). If a parasitoid did not show parasitism behaviour, the host was presented to another parasitoid in the group. In this way some parasitoids parasitised more hosts than others. For the L5 group, fewer hosts were available, leading to a reduction in the average number of parasitisms per individual parasitoid.

A separate group of 80 unparasitised larvae of *H. bicruris* were used as controls. All larvae were kept individually before and after parasitism in vials (30 ml Coulter Counter cups).

Measurements

Immediately following parasitism, each host larva was weighed to measure host mass and the number of previous parasitisms of the ovipositing wasp was recorded.

Several correlates of fitness were determined for *M. tristis*. Survival up to egression was recorded for each clutch. Development time was measured as the number of days between oviposition and egression. After egression, we measured secondary clutch size (parasitoid load), parasitoid pupal development time, survival up to succesfull eclosion, total clutch weight after cocoon formation, and we calculated average cocoon weight for each clutch.

To measure the effect of parasitism on host growth, 50 % of all host larvae were weighed every second day until egression of the parasitoid larvae to compare growth trajectories and calculate the maximum host growth after parasitism. For the same group of hosts, fresh weight of the diet given at each refreshment was recorded and its dry weight was calculated by determining the fresh weight/dry weight ratio based on a separate sample from the same diet. Diet remaining after refreshing was dried in an oven at 80 ºC for 3 days, when weight was stable, and weighed. Food consumption was calculated from the difference between the dry leftover weight and the calculated dry weight before feeding. Host weight after egression was measured for all hosts.

From the control group of (unparasitised) host larvae, development time, pupal weight, and survival up to pupation was measured. For approximately 50 % of this group, food consumption was determined and the growth trajectories were measured, just as for the parasitised caterpillars.

Weighing was undertaken using a Mettler Toledo (MT5) balance with 0.01 mg accuracy, whereas a Mettler AE 200 with 0.1 mg accuracy was used for caterpillars exceeding 30 mg.

Analyses

All data analyses were performed using the statistical package SPSS v. 10.0 (SPSS Inc, Chicago, IL) unless stated otherwise. Data from parasitised caterpillars were only included in analyses if successful parasitoid egression was observed, except for analyses of survival. Data from non-parasitised caterpillars were only used if larvae had pupated. Weight at parasitism was log-transformed before analysis.

Secondary clutch size (parasitoid load) was unaffected by host weight at parasitism but decreased with the number of previous parasitisms by a female wasp (two-way ANOVA ($F_{1,138}$ =5.313, $P<0.023$, interaction n.s.). Since the average number of previous parasitisms was confounded with host stage (smaller with wasps parasitising L5 than for other instars, see above), no attempt was made to analyse the clutch size responses of the parasitoid to variable host weights. However, irrespective of the cause of differences in parasitoid load among caterpillars, variation in parasitoid load may affect host and parasitoid development and hence its effect was included in analyses of host and parasitoid development.

Effects of host weight at parasitism and parasitoid load on parasitoid development time, average cocoon weight, host growth and total host food consumption were analysed by two-way ANOVA. To accommodate non-linear effects of host weight at parasitism, both linear and quadratic terms for this factor were included in the model. In the full models we included the main effects and the interaction effects between host weight and parasitoid load. Final models were developed by removing non-significant ($P>0.05$) interaction effects and subsequently non-significant main effects.

Effects of the average cocoon weight of parasitoid clutches on survival of parasitoid pupae to adult eclosion were tested using a generalised linear model with a binomial response variable and a logit link function (SAS procedure Genmod in SAS v 6.12, SAS institute, Cary, NC). Host survival for caterpillars parasitised in different instars was analysed using a G-test followed by multiple comparisons with sequential Bonferroni corrections (Rice 1989). We also included the survival from L1 to pupation of control hosts. A few female parasitoids apparently infected their hosts with some kind of infection (presumably bacterial), leading to the death of all hosts parasitised by these females within 48 hours after oviposition. We did not incorporate this mortality in our survival data.

In addition to the above analyses, we compared mean values of host and parasitoid development parameters among groups parasitised at the five different host instars using one-way ANOVA followed by post hoc multiple comparison tests (Tukey HSD or Dunnett3 test for unequal variances). This was done to facilitate conclusions about which instars should be parasitised to maximise parasitoid fitness correlates and to investigate up to which host instar parasitism can reduce host food consumption. For host development and food consumption, data of unparasitised caterpillars were included as a sixth group for comparison.

Results

Effects of host weight and stage on parasitoid development

Successful development of *M. tristis* from parasitism to egression was significantly lower in L1 caterpillars (57%) than for L2 and L4 instars, where survival was approximately 90 % (Table 3.1).

Parasitoid larval development time decreased with host weight at parasitism (Fig. 3.1 A, Table 3.2). Parasitoids developing in L5 hosts completed their larval development almost twice as quickly as conspecifics developing in L1 caterpillars (Table 3.1). Variation in larval development time was larger in instars L1 and L2 than in instars L3–L5. In L1 hosts, larval development time ranged from 15 to 48 days, whereas in L5 it

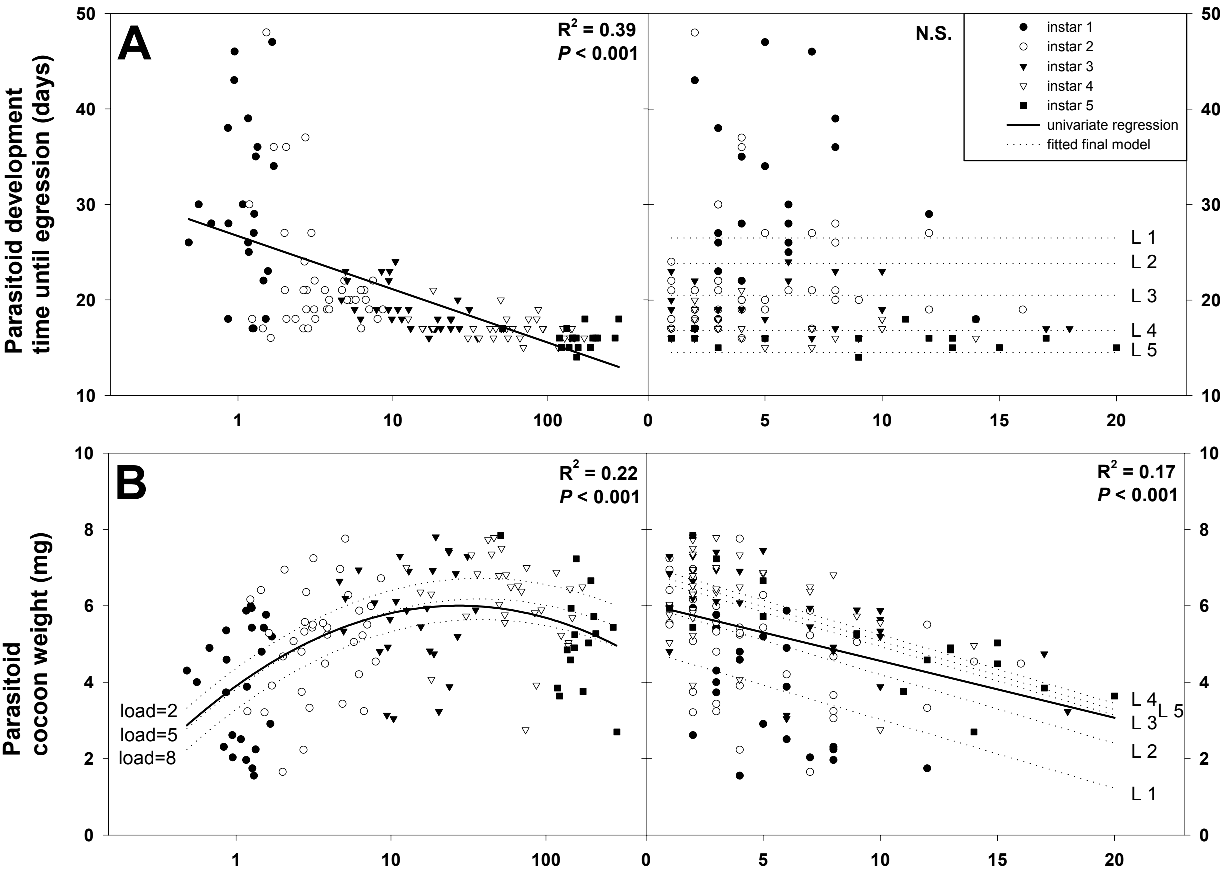

Figure 3.1 Effects of host weight at parasitism and parasitoid load on parasitoid larval development time (A) and average parasitoid cocoon weight (B). Solid lines represent univariate regression. Broken lines represent fitted values based on parameter estimates from two-way ANOVAs of effects of host weight and parasitoid load. Fitted lines are plotted for three levels of parasitoid loads (clutch size is 2, 5, or 8) and for the average weights of the five instar groups (L1-L5). Adjusted R^2 and *P*-values are indicated for the univariate regression.

ranged from 13 to 19 days (Fig. 3.1 A). Parasitoid load did not have a significant effect on larval development time (Fig. 3.1 A, Table 3.2). Parasitoid pupal development time was not related to host weight at parasitism nor to parasitoid load (two-way ANOVA, interaction and main effects not significant).

Average cocoon weight increased with host weight at parasitism to a maximum at around 25 mg, corresponding to instar stage L4, and declined again slightly in more heavy hosts (Fig. 3.1 B, Table 3.2). Parasitoids from L1 hosts attained maximum weights of 4 mg, whereas parasitoids from L4 hosts were on average 2 mg heavier, reaching up to 6 mg in weight (Table 3.1). Average cocoon weight decreased with higher parasitoid loads (Fig. 3.1 B, Table 3.2). Average cocoon weight per clutch had a significantly positive effect on the number of parasitoid larvae per clutch that survived until adult eclosion (Fig. 3.2). It did not affect the average time between pupation and adult eclosion of parasitoids (linear regression, n=115, *P*=0.14).

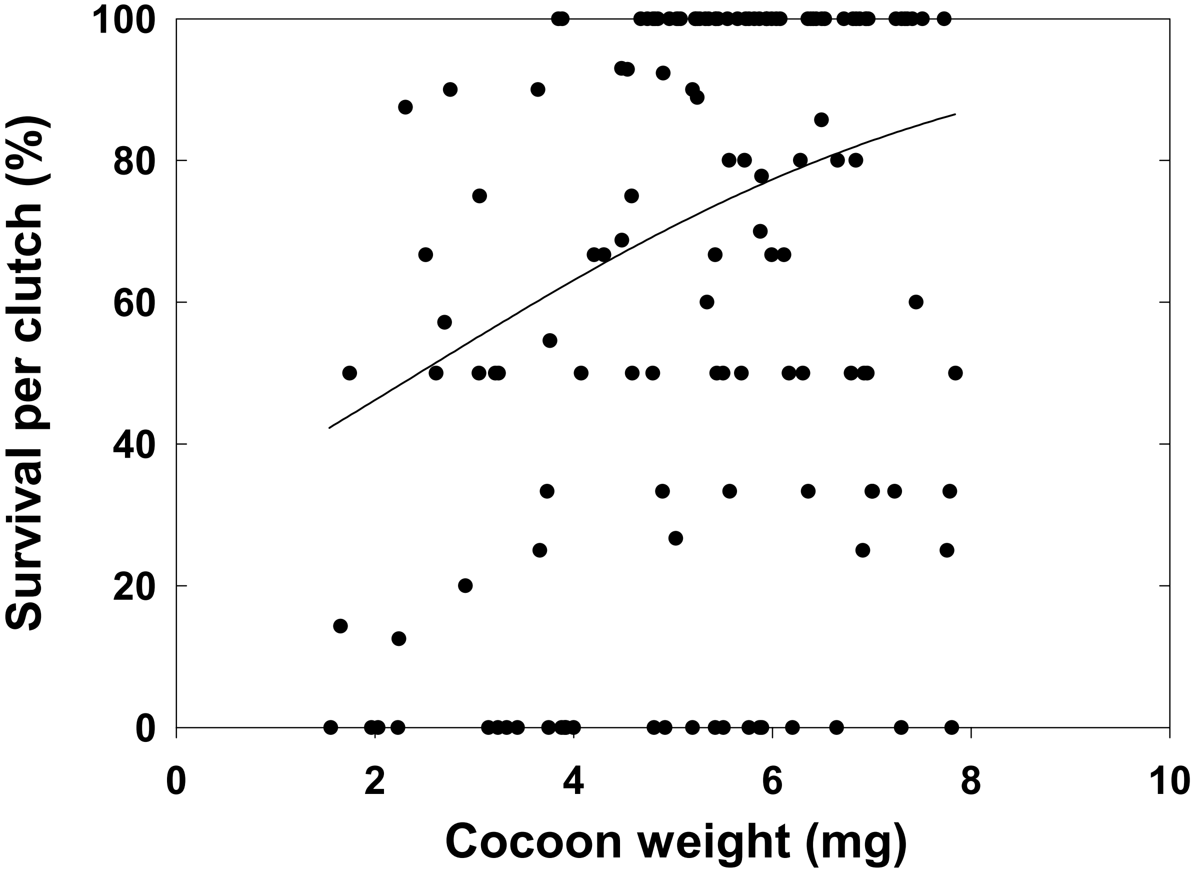

Figure 3.2 Effect of average cocoon weight of a clutch and the survival of pupae from that clutch to adult wasps (generalised linear model, *P*<0.001, n=140).

Table 3.1

	Instar 1	Instar 2	Instar 3	Instar 4	Instar 5	Controls
Parasitoid measurements						
% Hosts surviving until eggression	57.1 (42) A	89.4 (47) B	81.1 (37) AB	87.2 (39) B	76.0 (25) AB	87.8 (82) B
Larval development time (days)	29.7 ± 1.9 (23) E	22.5 ± 1.0 (42) D	18.9 ± 0.4 (30) C	17.0 ± 0.2 (34) B	15.9 ± 0.3 (18) A	
Pupal development time (days)	12.7 ± 0.4 (15) B	12.4 ± 0.2 (33) B	11.5 ± 0.2 (21) A	12.2 ± 0.2 (29) AB	12.3 ± 0.2 (18) AB	
Average cocoon weight (mg)	3.96 ± 0.31 (24) A	4.98 ± 0.23 (39) B	5.78 ± 0.26 (28) BC	6.17 ± 0.20 (32) C	4.84 ± 0.41 (18) AB	
Clutch size	4.79 ± 0.52 (24) AB	4.85 ± 0.58 (41) AB	5.87 ± 0.82 (30) AB	3.85 ± 0.54 (34) A	8.95 ± 1.38 (19) B	
Previous parasitisms	3.54 ± 0.37 (24) BC	3.85 ± 0.42 (41) C	3.00 ± 0.31 (30) BC	2.41 ± 0.22 (34) AB	1.63 ± 0.17 (19) A	
Host measurements						
Age at parasitism (days)	4.08 ± 0.82 (24)	7.76 ± 0.22 (42)	11.27 ± 0.22(30)	17.47 ± 0.31 (34)	23.78 ± 0.83 (19	
Weight at parasitism (mg)	1.13 ± 0.067 (24)	3.80 ± 0.33 (39)	15.26 ± 1.52(30)	73.54 ± 7.60 (33)	164.1 ± 13.36(18)	
Lifetime (days)	33.8 ± 1.9 (23) ABC	30.3 ± 1.1 (42) A	30.17 ± 0.4 (30) A	34.5 ± 0.4 (34) B	39.5 ± 0.9 (18) C	35.8 ± 0.6 (72) B
Maximum weight (mg)	128.3 ± 12.4 (12) A	178.3 ± 20.2 (25) AB	225.8 ± 20.2(15) BC	260.3 ± 14.9 (19) C	301.9 ± 15.2 (8) C	464.4 ± 6.3 (43) D
Weight after egression (mg)	57.7 ± 5.9 (22) A	85.9 ± 7.3 (40) B	112.1 ± 6.5 (28) B	166.0 ± 7.5 (34) C	164.6 ± 6.8 (18) C	304.0 ± 3.7 (72) (pupal weight)
Max. weight gain after parasitism (mg)	127.1 ± 12.4 (12) A	174.6 ± 19.9 (25) AB	211.0 ± 20.1(15) B	196.8 ± 14.0 (19) AB	142.1 ± 24.0 (8) AB	
Food consumption (mg)	277.9 ± 55.0 (10) A	559.1 ± 71.1 (23) AB	633.2 ± 83.9(15) BC	641.2 ± 47.4 (18) BC	922.7 ± 108.7(8) C	1692.0 ± 44.7(40) D

Table 3.1 (Continued) Growth and development related parameters for the parasitoid *M. tristis* and its host *H. bicruris,* given separately for hosts parasitised in different stages (instar 1-5) and unparasitised controls. Values are averages ± 1 s.e. Numbers of observations in parentheses (changes per instar are due to mortality and missing data). Different letters within a row indicate significant differences ($P<0.05$) between host stages (one-way ANOVA with Tukey HSD post hoc multiple comparisons tests or Dunnett3 tests for unequal variances). Host survival was compared with multiple Fisher exact tests (survival of controls was measured from L1 until pupation).

Table 3.2 F-values from ANOVA analyses for different host and parasitoid parameters. Values are obtained from the final models after non-significant interaction and main effects were removed (in that order) from the full models. Number of observations (n) and adjusted R^2 of the final models are given. *** = $P<0.001$ (see Figs. 3.1 and 3.2 for a graphical presentation of the final models).

	d.f.	Host weight after egression (n = 136)	Max. host weight gain (n = 79)	Host food consumption (n = 74)	Parasitoid development time (from oviposition to egression) (n = 142)	Parasitoid cocoon weight (n = 136)
Host weight at parasitism	1	199.959***	35.108***	24.499***	90.707***	46.986***
Parasitoid load	1	32.313***	37.021***	38.887***	-	58.159***
Host weight at parasitism^2	1	-	14.755***	-	-	24.754***
Host weight × parasitoid load	1	35.438***	-	-	-	-
Host weight^2 × parasitoid load	1	-	15.653***	-	-	-
Final model R^2		0.68	0.50	0.48	0.39	0.46

Effects of parasitism on host growth

Parasitism significantly affected host growth and development (Fig. 3.3, Table 3.1). The maximum weight of parasitised host larvae was considerably lower, and was reached 1-2 weeks earlier than the maximum weight of unparasitised larvae (Fig. 3.3, Table 3.1). Parasitised L1 hosts reached only about 25 % of the maximum weight of unparasitised caterpillars, whereas growth of parasitised L5 hosts was arrested at about 66 % of the maximum weight of unparasitised caterpillars (Table 3.1). Furthermore, in 29 % of the hosts parasitised as L1, egression took place as L3 (one individual) and L4. In L2 hosts 12 % egressed as L4, in all others egression took place as L5.

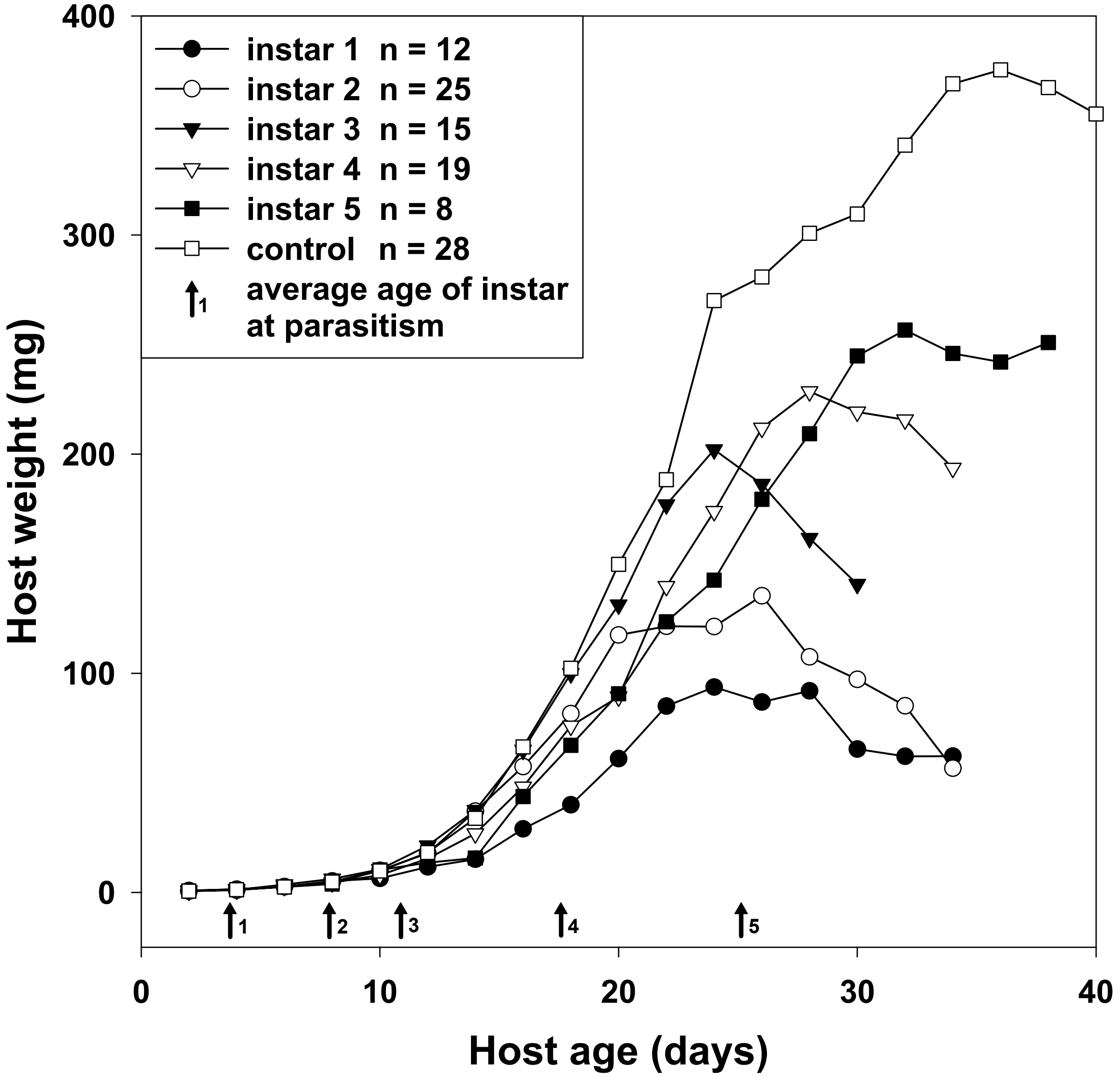

Figure 3.3 Growth curves of hosts parasitised in different larval stages (instar 1-5) and non-parasitised (control) hosts.

Lifespan of host larvae was also affected by parasitism. Host larvae that were parasitised as L2 and L3 lived on average 5 days shorter, whereas host larvae parasitised as L5 lived 4 days longer than unparasitised caterpillars (Table 3.1).

The weight of the caterpillars after parasitoid egression was positively correlated with host weight at parasitism (Fig. 3.4 A, Table 3.2), i.e., parasitoid larvae from ovipositions in different host stages did not egress at the same host weights. Parasitoid load also affected the weight of the host after egression (Fig. 3.4 A, Table 3.2). However, a significant interaction effect (Table 3.2) indicates that parasitoid load has a more strongly positive impact on the weight in smaller than in larger hosts (Fig 3.4 A).

Analyses of the maximum weight gain after parasitism also revealed that host growth was reduced by parasitism. The weight gain of all parasitised caterpillars was less than their potential weight gain if unparasitised (average maximum weight of unparasitised caterpillars minus the weight at parasitism) (Fig. 3.4 B, Table 3.1, t-tests, all $P<0.001$). The maximum weight gain of the host after parasitism was dependent on host weight at parasitism and on parasitoid load (Fig. 3.4 B, Table 3.2). Weight gain increased with parasitoid load but the significant interaction effect shows that this effect is stronger in hosts with smaller weights.

The survival rate of parasitised caterpillars (i.e., up to succesfull egression) was lower than for unparasitised caterpillars (i.e., up to pupation) for most instar stages. This can be concluded from a comparison of the survival rates of different host instars when they are parasitised (Table 3.1) and the survival of hosts of the corresponding stage when they are not parasitised (L1 to L5, respectively: 87.8, 92.3, 93.5, 97.3 and 97.3%, comparisons with Fisher exact tests respectively: $P<0.01$, n.s., $P=0.04$, $P=0.05$, $P<0.01$).

Effects of parasitism on host food consumption

Parasitised hosts consumed significantly less diet than control caterpillars during their development (Fig. 3.4 C, Table 3.1). Total food consumption was found to be lowest in caterpillars that were parasitised as L1, consuming only 300 mg of food, while unparasitised hosts consumed up to 1700 mg to complete development. Even the largest parasitised L5 caterpillars consumed only about 50 % of this amount (Table 3.1). The amount of diet consumed by *H. bicruris* larvae was not only dependent on host weight at parasitism but also increased with parasitoid load (Fig. 3.4 C, Table 3.2).

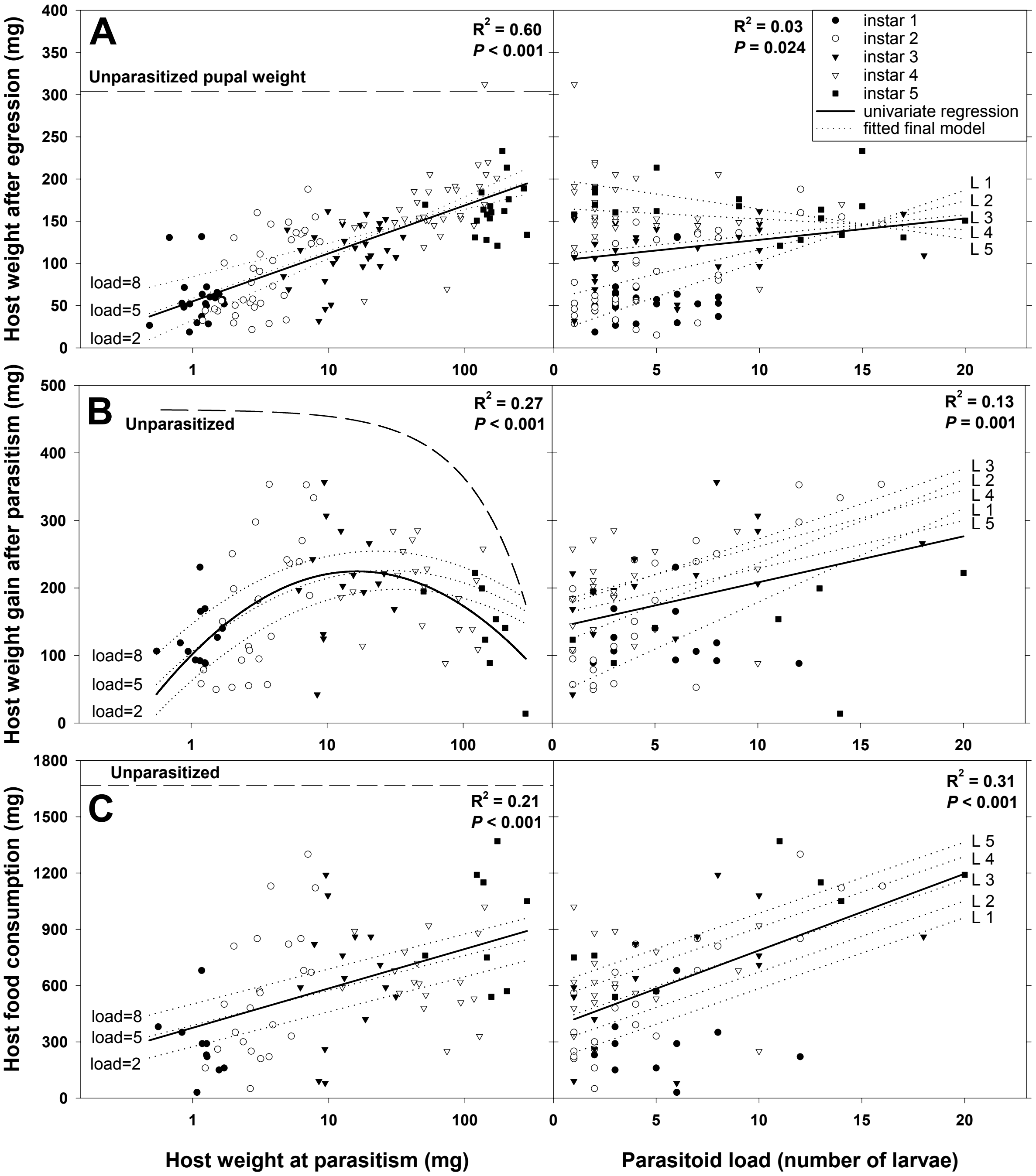

Figure 3.4 Effects of host weight at parasitism and secondary clutch size on host weight after egression (A), host growth (B), and host food consumption (C). Solid lines represent univariate regression. Broken lines represent fitted values based on parameter estimates from two-way ANOVAs of effects of host weight and parasitoid load. Fitted lines are plotted for three levels of parasitoid loads (clutch size of 2, 5, or 8) and for the average weights of the five instar groups (L1-L5). Non-parallel broken lines indicate significant interaction effects. Adjusted R^2 and P-values are indicated for the univariate regression.

Discussion

The results of this investigation reveal that, in the interaction between *H. bicruris* and *M. tristis*, parasitised larvae from all instars and across all weights at parasitism consume less diet and exhibit reduced growth compared to unparasitised larvae. However, the effects are most pronounced when hosts of small weights are parasitised. Many solitary and gregarious koinobiont parasitoids have been reported to manipulate host growth during parasitism or to adjust their own feeding behaviour in response to changing host conditions (Pennacchio *et al.* 1993, Balgopal *et al.* 1996, Harvey 2000), which is presumably an adaptation that stabilises variation in resource availability at oviposition to the parasitoid larvae. Several studies with gregarious koinobiont parasitoids have shown that parasitised hosts exhibit accelerated growth or attained larger terminal weight than unparasitised larvae (Rahman 1970, Schopf and Steinberger 1996). By contrast, few studies have reported a reduction in host growth (van der Meijden and Klinkhamer 2000), as is demonstrated in this study.

Host growth was reduced by *M. tristis* in all instar stages, but maximum size attained varied between host instars by almost a factor three. Growth reduction was strongest in hosts of small weights at parasitism. In these early parasitised hosts, parasitoid egression did not take place at similar host weight and stage as in later parasitised hosts, but at an earlier stage and lower weight, a phenomenon also reported in other microgastrine braconids (Beckage and Riddiford 1983, Harvey 2000).

Although the growth of parasitised hosts was not stimulated compared to unparasitised larvae, we found evidence that the maximum weight of the host parasitoid complex was positively correlated with parasitoid load, at least in small hosts. The gain in weight with increasing parasitoid loads was accompanied by an increase in the amount of food consumed by the host. For several other species of gregarious koinobionts brood size is reported to enhance host growth, thereby maintaining a fairly constant supply of resources to the parasitoid offspring (Sato and Tanaka 1984, Harvey 2000). This may help to reduce scramble competition and allow parasitoid larvae to complete their development normally. However, growth stimulation cannot always prevent sibling competition when parasitoid loads exceed a certain threshold, thus resulting in smaller adult parasitoids (Alleyne and Beckage 1997, Harvey 2000). This was also observed in *M. tristis*, where cocoon weight decreased with parasitoid load.

Host weight at parasitism significantly affected development time of the parasitoid. In caterpillars parasitised as L1, completion of development took almost 30 days, whereas in L5 hosts, it took less than 16 days. The average age of L5 hosts at parasitism was already 24 days and host larval lifespan was prolonged by 4 days compared to unparasitised caterpillars that completed their development in 36 days. This indicates that the development of L5 hosts is arrested prior to pupation. Many solitary and gregarious koinobionts are also known to arrest host growth in a similar fashion (Jones and Lewis 1971, Tagawa *et al.* 1982, Sato and Tanaka 1984, Dover and Vinson 1990). Factors injected by the female parasitoid during the oviposition sequence (e.g. polydnaviruses, venoms, etc.) (Dushay and Beckage 1993) may prevent the host from reaching a condition in which the parasitoid cannot develop successfully.

Several solitary koinobionts attacking concealed hosts are known to suspend their development in small hosts until the host reaches an appropriate size or nutritional value (Corbet 1968, Harvey *et al.* 1994, Harvey 2000). Consequently, parasitoid larval development time may be highly variable whereas the size of emerging wasps is quite constant (Harvey *et al.* 2000). In these parasitoids, it is likely that immature survival is independent of development time, thus allowing for the benefits of increased size to be realised (Harvey and Strand 2002). Similarly, the development of *M. tristis* was prolonged when developing in early instar hosts, which probably allowed hosts and parasitoid larvae to attain a critical minimum viable size for the completion of parasitoid development. However, the size of emerging wasps was not constant. Even the 12 day increase in development time of parasitoids developing in L1, as compared to parasitoids developing in L4, was insufficient to allow the parasitoids to attain the same weight as parasitoids developing in L4 hosts. This suggests that beyond a delay in development time that is necessary to attain a critical size, the disadvantage of a further delay in development time might become more important than the advantage of a further increase in size. (Mackauer and Sequeira 1993). Like in other gregarious koinobionts, larger parasitoid loads did not lead to an extra delay of development (Schopf and Steinberger 1996), as has been observed in some ectoparasitoids (Harvey *et al.* 1998).

The consequences of a delay of parasitoid larval development on parasitoid fitness have not been well studied. Not only might there be negative effects from a longer generation time, but, as predicted by the slow-growth-high-mortality hypothesis (sensu Clancy and Price (1987)), a longer development time might also decrease the chance of survival due to a prolonged window of vulnerability to predators, pathogens or hyperparasitoids (Benrey and Denno 1997). During early instars, *H. bicruris* is normally concealed within the closed seed capsules of its host plant, suggesting that delayed development might not necessarily incur an extra cost in terms of predation or hyperparasitism.

In contrast to the paucity of data on consequences of delayed development, many studies have discussed the importance of body size or weight of parasitoids on fitness parameters. Body size is often strongly positively related with reproductive success because larger females live longer, produce more eggs, and exploit hosts more effectively than smaller conspecifics (Visser 1994, Harvey *et al.* 2001). In this study, cocoon weight had an overall positive impact on survival of pupae to the adult stage. Also the dispersal ability of parasitoids may be strongly positively correlated with size (Ellers *et al.* 1998), a factor which might be of importance in a system where hosts are distributed in meta-populations with large inter-patch distances, such as the *S. latifolia-H. bicruris-M. tristis* system.

Our experimental setup was not appropriate for testing whether *M. tristis* adjusts its clutch size with host weight due to the confounding factors of age and the variable experience of the female parasitoid. Clutch size of *M. tristis* was strongly negatively affected by the number of previous parasitisms of the individual parasitoids, whereas no additional effects of host weight at parasitism could be detected. Although this suggests that *M. tristis* does not adjust clutch size to the weight of its host *H. bicruris*, more rigorous testing is needed to substantiate this conclusion. Evidence for clutch size adjustment in koinobionts is scarce. Sato and Tanaka (1984) found no clutch size adjustment in the gregarious koinobiont *Cotesia kariyai*. Similarly, Harvey (2000) reported that clutch size in *C. glomerata* was similar in different host instars and species that varied by many factors in size at oviposition. On the other hand, Schopf and Steinberger (1996) found an increase in clutch size with host size in *Glyptapanteles liparidis* as did Sato *et al*. (1986) for *Cotesia ruficrus* and *C. kariyai*.

Host weights corresponding to L4 instars seem to be optimal for parasitoid development of *M. tristis*, because parasitoid larvae developing in this instar complete their development more quickly than larvae developing in early instars, experience lower mortality, and have a larger mean cocoon weight than larvae developing in either lighter or heavier hosts. Parasitism of heavier (L5) hosts resulted in a decrease in average parasitoid cocoon weight indicating that hosts might have entered a prepupal stage that negatively affects the growth of the parasitoid.

In the present system, development and host selection of *M. tristis* represent a case of “optimal synchronisation”. Although M. tristis is clearly able to develop on all host instars, it develops most successfully on later host stages. In nature, it is likely that *M.*

tristis only encounters L4 and L5 instars with high frequency, because earlier instars are concealed in the developing seed capsule of their food plant, *S. latifolia*. This is supported by a field study (Biere *et al.* 2002), in which parasitism rates of *H. bicruris* were found to be much higher in later than in early instars (46% for L5, 17% of L4 and none in smaller instars).

Related species, like *C. glomerata*, that attack exposed hosts, actually prefer early instars over later instars, presumably because later instars more actively resist parasitism and may actually be able to kill or debilitate parasitoids attempting to oviposit in them (Dover and Vinson 1990, Gross 1993). In addition, larger hosts are often less physiologically compatible than younger hosts (Dover and Vinson 1990, Strand and Pech 1995, Harvey 2000).

Many idiobiont and solitary koinobiont parasitoids are known to decrease host consumption considerably, compared with healthy hosts, and are therefore considered good species for biological control programmes (Huebner and Chiang 1982, Rohlfs and Mack 1983). By contrast, a number of gregarious koinobiont species are known to increase the host's total food consumption (Rahman 1970, Slansky 1978, Beach and Todd 1986). However, this study reveals that gregarious koinobionts can also decrease host food consumption. Healthy caterpillars of *H. bicruris* are able to consume all developing seeds of approximately five seed capsules, containing several hundreds of seeds, during their lifetime (Brantjes 1976b). Although larger clutch sizes do lead to higher consumption rates, the results clearly show that the amount of food eaten by parasitised L4 or L5 caterpillars is considerably lower than food consumption by unparasitised hosts.

This implies that parasitism can have a direct positive effect on host plant fitness. Not only will populations of *S. latifolia* benefit from the presence of *M. tristis* by reducing the number of herbivores in the next generation, but they will also benefit directly due to the reduction of the damage by a single herbivore (van Loon *et al.* 2000). Van der Meijden and Klinkhamer (2000) questioned whether plants would benefit when attracting specialist gregarious koinobiont parasitoids through indirect defence mechanisms like volatiles (Vinson 1999). Our study system appears to be a case where this might be truly beneficial. However, whether *S. latifolia* attacked by *H. bicruris* actually produces specific volatiles that enhance attraction of *M. tristis* and whether this enhances plant performance is as yet unknown.

In summary, this study has reported that *Microplitis tristis* can develop successfully in all host sizes of *Hadena bicruris*. However, in small instars there is an increase in development time and a decrease in parasitoid size, resulting in a smaller chance of survival until eclosion. Although clutch size has a positive effect on the growth of the host larvae it cannot prevent severe scramble competition in small hosts. Heavier hosts are more optimal for *M. tristis* from a physiological perspective and parasitism of L4 appears to maximise most of the studied fitness correlates. This clearly suggests an adaptation to the life history of the host, as small hosts are usually concealed and unavailable for parasitism. Because parasitism decreases the amount of food consumed by the host, the parasitoid can have a direct positive impact on populations of the host plant.

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Chapter 4

Dispersal patterns of three parasitoids and their specialist herbivore host in a fragmented landscape

with Saskya van Nouhuys, Dirk-Jan van Leeuwen and Arjen Biere

Abstract

Good dispersal ability is essential for species to persist in fragmented habitats. Habitat fragmentation can disrupt communities if interacting species have different dispersal abilities. For instance, if parasitoids disperse less well than their herbivorous hosts, isolated plant patches may experience more herbivory due to the absence of the third trophic level. The dispersal behaviour of parasitoids is not well known. Community studies suggest a limited dispersal ability, in the order of several tens of meters, much smaller than that of the hosts, while species-oriented studies suggest dispersal abilities of several kilometres.

In this study the distribution patterns of three parasitoid species and their host, *Hadena bicruris,* a specialist herbivore of *Silene latifolia*, were determined in large scale fragmented natural plant patches. Additionally, experimental plant patches were established to study the colonisation abilities of the species at different distances from source populations.

The distribution patterns indicate that the presence of the herbivore and two of the parasitoids, the gregarious specialist *Microplitis tristis* and the gregarious generalist *Bracon variator* were not affected by patch isolation, while the solitary specialist *Eurylabus tristis* was. In contrast with the herbivore, the presence of all parasitoid species declined with the size of a plant patch, most strongly for *E. tristis.* The colonisation experiment confirmed that the herbivore and *M. tristis* are good dispersers, able to travel at least 2 km within a season. *B. variator* showed intermediate colonisation ability and *E. tristis* showed very limited colonisation ability at this spatial scale.

Characteristics of the parasitoid species that could contribute to differences in their dispersal abilities are discussed.

Introduction

Species with intermediate dispersal abilities are able to colonise suitable habitat patches and recolonise patches where they have gone locally extinct, thus maintaining the balance between colonisation and extinction in a patchy environment. This is one of the basic concepts for stable metapopulations in the classical metapopulation models of Levins (Levins 1969, Hanski and Gilpin 1996, Hanski 1999). Extension of the classical metapopulation theory recognises important processes in metapopulations other than recolonisation that are consequences of dispersal and migration (Hanski 1999). For instance, immigration from other patches can prevent local extinction in small patches by increasing the local population size (the so called rescue effect (Brown and Kodric-Brown 1977)) or preventing Allee (Courchamp *et al.* 1999) and inbreeding effects (Frankel and Soulé 1981). Although it is debated how applicable the metapopulation approach is to species in general, it is recognised that many species live naturally in a patchy environment and that dispersal processes often play a key role in regional persistence (Harrison 1994, Halley and Dempster 1996, Hanski 1999).

Currently, the scale of human induced habitat fragmentation imposes even a greater demand on dispersal ability for species to persist. Not only are habitat sizes reduced due to deforestation, agriculture, urbanisation, etc., leading to a decrease in population sizes and potential dispersers, but also the distances that have to be crossed between fragments become larger (Saunders *et al.* 1991, Groombridge 1992, Henle *et al.* 2004).

More importantly, habitat fragmentation can disrupt whole communities (Wilcove *et al.* 1986, Quinn and Harrison 1988, Baur and Erhardt 1995, Tscharntke *et al.* 2002) because the chance of a particular species persisting in a habitat patch can be strongly affected by the presence or absence of other species, like for instance, flowering plants whose reproduction depends heavily or completely on the presence of their pollinators (Steffan-Dewenter and Tscharntke 1999) or plant-herbivore-parasitoid systems, where all three trophic levels can influence each others population dynamics. In several studies it has been shown that fragmentation affects the number of species in different guilds and trophic levels, potentially disrupting communities (Kareiva 1987, Kruess and Tscharntke 1994, Zabel and Tscharntke 1998, Golden and Crist 1999, Komonen *et al.* 2000).

Few empirical studies have focused on the dispersal abilities of parasitoids in natural systems and the consequences for plant-herbivore-parasitoid interactions in fragmented habitat. In general, it is noted that fragmentation leads to increased pest incidence, perhaps because predatory insects and parasitoids are not able to disperse as well as their herbivores (Roland 1993). In contrast, Brodmann *et al.* (1997) showed that pest outbreaks can be stopped by parasitoids that disperse well compared to their hosts in a fragmented habitat on a scale of several 100 meters. However, several studies show that isolated plant patches are more easily colonised by herbivorous insects than their predators or parasitoids even at the scales of a few hundred meters (Kruess and Tscharntke 1994, Zabel and Tscharntke 1998, Komonen *et al.* 2000), indicating that the third trophic level insects disperse or colonise less well than the second trophic level insects, and are thus more strongly affected by habitat fragmentation. Still, actual dispersal ability and colonisation patterns of insect species in general (Stein *et al.* 1994), and particularly parasitoids in relation to their hosts, are not well understood (Jones *et al.* 1996), partly due to logistic and practical difficulties (Hagler *et al.* 2002).

Studies focusing on the dispersal of specific parasitoid and host species that are performed in a continuous environment, at small distances or over a short time span, indeed show that only a very small proportion of parasitoids released in a suitable habitat disperse up to the maximum measured distances of 100 meters (Ellers *et al.* 1998, Fournier and Boivin 2000, Sallam *et al.* 2001, Hagler *et al.* 2002), which is less than assumed for their host species. In contrast, Jones *et al.* (1996) found that several parasitoid species dispersed better, i.e. up to 50 m, than their tephritid host, which could disperse up to 15 m within a habitat patch during its life.

However, dispersal patterns of parasitoids in relatively homogeneous agricultural areas where they did not occur naturally, after introduction for biological control, indicate much larger dispersal ability of parasitoids (Godfray 1994, Jones *et al.* 1996, Hastings 2000). Many of these studies show rates of spread for parasitoids on the order of a few to tens of kilometres a year over a few generations, with only a few exceptions where a parasitoid species was found to disperse only several 100 meters (see refs. in: Godfray 1994, Jones *et al.* 1996, Goldson *et al.* 1999), but in these studies information about the dispersal abilities of the hosts is mostly lacking.

Studies on the dispersal of hosts and parasitoids in a patchy, fragmented environment are scarce. Only a few studies have focused on colonisation patterns of herbivores and parasitoids in existing fragmented patchy habitats or experimental habitat patches over different distances, interspaced with unsuitable matrix on a scale of several kilometres (but see Cronin (2004) for a study on a scale of a few tens of meters). Van Nouhuys and Hanski (2002) found that the parasitoid *Hyposoter horticola,* could colonise patches up to several kilometres from a source population within a year, whereas the second parasitoid *Cotesia melitearum,* could not colonise habitat patches further than 1 km during the same period (see also Lei and Hanski 1998). The host herbivore *Melitea cinxia,* showed an intermediate dispersal ability. Antolin and Strong (1987) found that the very small egg parasitoid *Anagrus* and its host regularly disperse 1 km or more, even to off-shore islets, crossing the water. Dempster *et al.* (1995a) studied colonisation of plant patches by several herbivorous insects and their parasitoids and found that all species were highly mobile, colonising patches up to 800 m from a source of immigrants and found movements of up to 1-3 km or even outside the study area for several parasitoids and their hosts within a year (Dempster *et al.* 1995b).

These large scale studies on individual parasitoid species show that many have a potential ability to disperse over large distances of several kilometres, but the majority of the community studies suggest that parasitoids have a smaller dispersal ability than their hosts and can be affected strongly even at scales of a few hundred meters (Zabel and Tscharntke 1998, Kruess and Tscharntke 2000). Several studies suggest that different species of parasitoids respond to different scales of fragmentation, even if associated with the same host species (Roland and Taylor 1997, Doak 2000).

In this study we investigate the impact of large scale fragmentation of patches of the plant *Silene latifolia* on the distribution pattern of the specialist noctuid herbivore moth, *Hadena bicruris* and three of its parasitoids in a rural landscape. The natural distribution patterns of all these species are compared with their colonisation patterns in experimental plots with different degrees of isolation.

It is expected that the noctuid moth is a very good disperser, as has been shown for similar noctuids (Schneider 1999), but that the parasitoids have lower dispersal abilities. Therefore we expect that at any given moment, the parasitoids are more frequently absent from isolated and small plant patches due to a lower recolonisation rate after local extinctions (Hanski 1999). Similarly, the herbivore should colonise more experimental plant plots located at longer distances from a source population than will the parasitoids. The three species of parasitoids are expected to be differentially affected by fragmentation, depending on their biology (i.e. small specialist gregarious, large specialist solitary, small generalist gregarious). It is expected that the large parasitoid disperses greater distances than the small ones and that its presence is therefore less affected by habitat fragmentation (Tscharntke and Brandl 2004). The generalist is not expected to be strongly affected by fragmentation because it probably occupies surrounding habitats. Life-history characteristics that might explain differences in dispersal and colonising distances among parasitoids, and the possible consequences of habitat fragmentation in the system are discussed.

Material and methods

Study species

Plant

Silene latifolia Poiret (= *S. alba* (Miller) Krause = *Melandrium album* (Miller) Garcke), the white campion, is a dioecious weedy short lived perennial occurring in open disturbed habitats such as fallow fields, field margins and roadsides (Goulson and Jerrim 1997). It grows in chalky sandy soil. It is native to Eurasia, and has been introduced to North America where it is considered a pest species (Wolfe 2002).

In north-western Europe *S. latifolia* flowers from the end of April until 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). Its flowers have white petals that usually open in the late afternoon. During the night flowers emit a strong scent attracting noctuid moths and hawkmoths (Jürgens *et al.* 2002a). Pollination in Europe is mainly performed by moths, with *H. bicruris* and *Autographa gamma* as most common visitors, although some hoverfly species visit the flowers during the day (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 Europe the most important herbivore consuming the developing seeds is the noctuid *H. bicruris* (Brantjes 1976b, Wolfe 2002)

Herbivore

Hadena bicruris Hufn. (Lepidoptera: Noctuidae), the lychnis, is a night-active moth with a wingspan of 30-40 mm. Caterpillars can be found in the Netherlands from May until October, but usually two peaks in June and at the end of August occur (pers. obs.), indicating that there are two overlapping generations. After pollination and nectarfeeding, the female moth may oviposit a single egg on the ovary of female flowers (Brantjes 1976b). Although it strongly prefers *S. latifolia,* it can use a few other closely related caryophyllaceous plant species (Wirooks and Plassmann 1999). However, no signs of *H. bicruris*, nor other *Hadena* species were observed on those caryophyllaceous plants present in the study area (pers. obs.).

Directly after hatching the first instar caterpillar (L1) chews a small hole in the side of the ovary, enters the young fruit, and feeds on the ovules and developing seeds. It will stay in the seed capsule (called primary capsule) until it is has consumed all the seeds, which usually occurs when the caterpillar has reached the fourth or fifth (last) (hereafter L4 and L5, respectively) instar. At this stage the caterpillar starts feeding on other seed capsules from the top (called secondary capsules). Late instar caterpillars are usually too large to fit entirely 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). At maturity, the larva leaves the food plant and pupates in the soil, after which it continues to develop or starts diapause depending on the season (Elzinga *et al.* 2002).

Parasitoids

Microplitis tristis Nees (Hymenoptera: Braconidae) with a wingspan of 3.5 mm is a small gregarious koinobiont endoparasitoid specialised on caterpillars of *H. bicruris* (Shenefelt 1978, Chapter 2). It usually attacks L4 and L5 caterpillars when they are too large to be protected by the seed capsule (Elzinga *et al.* 2003). The average clutch size in the field is around 18 (Elzinga *et al.* 2003). During development, larvae of *M. tristis* feed primarily on host haemolymph. When larval growth is complete, mature parasitoid larvae egress from the caterpillar, usually in the soil but occasionally in an empty seed capsule, by chewing a hole through the host's cuticulum. The parasitoid larva then spins a cocoon next to the caterpillar and pupates or goes into diapause as a prepupa. The caterpillar remains alive for up to several days, but eventually perishes without successfully completing development. Parasitised caterpillars can be found throughout the summer (Elzinga, pers.obs.), suggesting that several generations may overlap during a year.

Eurylabis tristis (Gravenhorst) (Hymenoptera: Ichneumonidae) is a large solitary larval-pupal endoparasitoid with a wingspan of 16 mm, and is a specialist on *Hadena* species (Chapter 2). It attacks L4 and L5 caterpillars of *H. bicruris* but the parasitoid larva does not develop until the host is in the pupal stage. The adult parasitoid egresses from the host pupa approximately two weeks after pupation or after diapause (Chapter 2).

Bracon variator Nees (Hymenoptera: Braconidae) is an extremely polyphagous gregarious ectoparasitoid with a wingspan of 4 mm. It is known as a parasitoid of larvae of very different groups of insects (Chapter 2). However, we do not know how generalised in host and habitat use it is in our study area. *B. variator* paralyses its host before depositing eggs on it. It usually attacks L4 and L5, rarely L3 of *H. bicruris*. The clutch sizes found in the field on *H. bicruris* range from 1 to 11 with an average of 4.5 ± 0.3 s.e. (Chapter 2).

Study area

Our study was performed 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 near the city of Gorinchem (Fig. 4.1). We searched for *S. latifolia* in floodplains and along riverdikes with different types of management, soil and elevation. The floodplains are usually submersed in winter. While a large fraction of the area is used for nature conservation where only herds of wild horses and cattle graze, other parts are used for agriculture (mainly haymaking) or for industrial purposes (e.g. brick factories). *S. latifolia* patches mainly grow on elevated sandy places, but also occur in disturbed areas near industrial sites and along roadsides and field margins. Many patches of *S. latifolia* also grow on the riverdikes, which are mostly mown twice a year. The surrounding area was also searched, but it was found to be mostly unsuitable for *S. latifolia* due to heavy agriculture and less suitable soil. Due to the clear borders, and the linear structure which made it more efficient to search, the area was very suitable for this study.

Patch location

In May and early June 2000 and 2001 the whole area was scanned by foot and by bicycle to locate flowering plants. When flowering is at its peak, the conspicuous white flowers are easily located. The minimum distance to distinguish between plant patches was 100 m. In every patch the coordinates of plants at the edges of the patches were recorded with a GPS system (GIS*backpack*, Commetius, Leiderdorp, NL). With these data the nearest neighbour distances (NND) as well as the distances between all patches were calculated.

Measurements

In every patch the number of reproductive plants (plants with flowers, seed capsules, or flower buds) was counted in August 2001, early June 2002 and early June 2003. At every census the plants were checked for eggs or signs of herbivory by *H. bicruris*. Damaged seed capsules were recognised by a small entrance hole at the side (a primary capsule) or by a large entrance hole at the top (a secondary capsule). Visual sightings of adults of any of the parasitoid species were also recorded at every visit.

In September 2001, June and September 2002, and June and September 2003 all plant patches were visited and caterpillars that were inside a secondary capsule or moving freely on the plant, were collected. Collected caterpillars were reared in the laboratory on artificial diet (Elzinga *et al.* 2002) to find out whether they were parasitised by any of the three parasitoid species. Because heavy sampling might disturb local population dynamics, caterpillars were collected maximum two times a year.

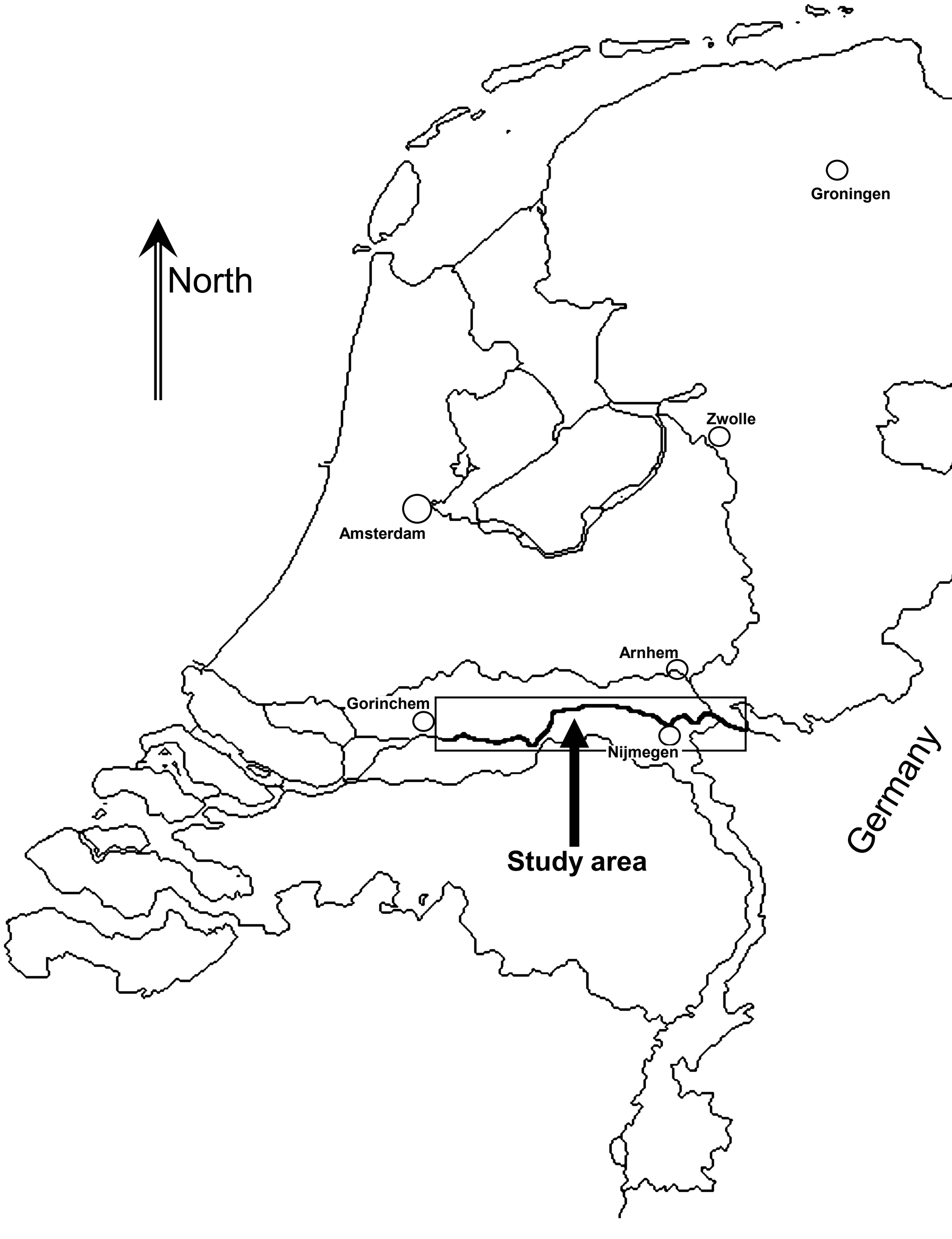


Figure 4.1 Map showing the location of the study area along the river Rhine and Waal in the Netherlands. The rectangle is 80 km wide.

Fragmentation estimates

Several parameters that are often used to indicate the level of fragmentation of a habitat were calculated. The two important factors related to fragmentation in this study are the size and the level of connectivity of the patches.

Because the densities of plants in the patches varied considerably, the number of reproducing plants was the most appropriate parameter for patch size. Patch size was calculated as the maximum number of reproducing plants counted in a year during the research period. Although changes in these numbers occurred from year to year, the overall pattern of fragmentation of patches was relatively constant over the study period. Only a few small patches (with less than three plants) disappeared during the period and a few new ones were discovered.

For the parasitoid, patch size might not be perceived through the number of plants but rather trough the number of herbivore hosts (van Nouhuys and Hanski 2002). Although the actual host numbers present in every patch were not determined, we expect that the number is highly correlated with the number of plants in a patch. Because all patches were searched with a constant effort it is expected that the number of collected caterpillars is correlated with the actual host numbers present, and the number of collected caterpillars in a patch was therefore used as a correlative estimate of patch size in the analyses of the distribution pattern of the parasitoids.

Connectivity of patches can be estimated in many ways (Moilanen and Nieminen 2002). In this study two types of estimates were used. First, estimates based on nearest neighbour distances (NND) were used. NND can be a good estimate of isolation in situations where the landscape has a linear structure (Moilanen and Nieminen 2002), as is to a large extent the case in this study along a river. To avoid extremely small populations affecting the estimate too strongly, not only the NND to the nearest plant patch with at least one female was used (NNDfem), but also the NND to the nearest patch with at least 10 plants present (NND10). As the presence of a parasitoid species is expected to depend also on the distance from a potential source population the NND to the nearest patch where the presence of a parasitoid species was observed was also used (NNDmicro, NNDeury, NNDbracon for *M. tristis*, *E. tristis*, *B. variator*, respectively).

As a second estimate of connectivity, a measure originally used in the incidence function model (Hanski 1999) was calculated. According to this model, the connectivity (S_i) of a patch i can be calculated as follows:

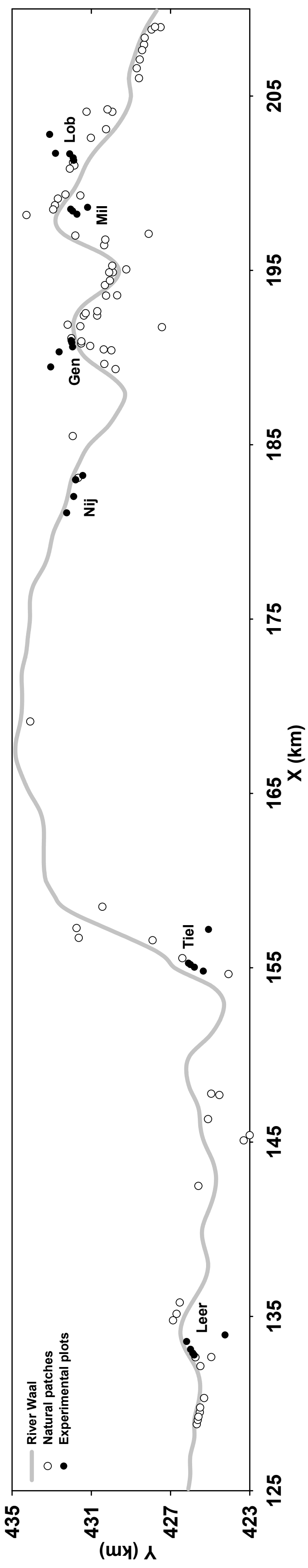
$$S_i = \sum_{j \neq i} p_j \cdot N_j \cdot e^{-\alpha d_{ij}}$$

where alpha (α) is a constant determining the effect of distance on isolation, d_{ij} is the distance between population i and j (in km) and N_j is the number of plants in population j (Hanski 1999). The p_j stands for presence and is either 1, when a species is present or 0 when a species is absent from a population j.

The constant alpha describes how steeply the number of migrants from patch j decline with increasing distance due to a.o. mortality (Hanski *et al.* 2000) and usually 1/alpha is considered as the average dispersal distance of a species (Moilanen and Nieminen 2002). Using mark-recapture data, alpha can be estimated (Hanski 1999) but because these data are not available for the species in this study, the appropriate values for alpha are unknown. Therefore S was calculated for a broad range of alpha's ranging from 0.2 to 50. Because this study is investigating fragmentation effects for each species separately, S was calculated taking into account either all plant populations ($p_j = 1$) (called S_{plant}) or taking into account only plant populations where a certain parasitoid species was observed ($p_j = 1$ or 0) (called S_{spec}).

Experiment

In 2002 and 2003 an experiment was performed to investigate the potential dispersal and colonisation abilities of the different species, using experimental plant plots. First, areas were selected, where it was possible to establish experimental plots at increasing distances, up to 2000 m from a natural source population, without coming within the same distances from other potential source populations and where at least the parasitoid *M. tristis* and the herbivore had been observed in 2001 or 2002. After selection of six areas, locations suitable for experimental plots at 125, 250, 500, 1000, and 2000 m were located. The specific locations were selected on the basis of the following criteria: accessability, relatively undisturbed by grazing or mowing, and suitable soil, i.e. not too wet. At every location a circle with the distance to the source as diameter was thoroughly searched to make sure that within that distance no other plant patches were present. Finally, 29 plots were established with one plot at every distance per area (except for a 2000 m plot at one location) (Fig. 4.2).



The plants in the plots were grown from seedlings in a garden in Heteren, The Netherlands. Flowers were removed daily to ensure that *H. bicruris* could not colonise the plants by laying eggs before the experiment. In July 2002 at each location 14 plants, 7 males and 7 females, were planted about 30 cm apart.

After 4 weeks, weekly censuses of the plots were started that continued until late September 2002, during which the plants were checked for eggs or signs of herbivory of *H. bicruris* and adult parasitoids. When available, L4 or L5 caterpillars visible on the plant or inside secondary seed capsules were collected and reared through in the lab. In early 2003 approximately half of plots had disappeared and were re-established and all plots were visited regularly during the rest of that year.

Analyses

We tested whether patch size and isolation could explain our presence/absence observations of the different species in the natural plant patches using generalised linear models (GLM) with a binomial distribution and a logit-link function (McCullagh and Nelder 1989) containing one or two explaining variables, A (= patch size [number of collected caterpillars or plant patch size]) and B (= isolation [NND or S]) in the freely available statistics software package R v.1.8.1 (Venables *et al.* 2003) for all patches where caterpillars were collected. As the number of collected caterpillars was expected to be correlated with the possibility that a parasitoid was encountered, analyses were performed

Figure 4.2 Map showing the natural (open circles) and experimental patches (filled circles) of *S. latifolia* in the study area. Names of the source locations are shown.

with the number of collected caterpillars as a covariate, i.e. the number of caterpillars was included in the models as an offset value (McCullagh and Nelder 1989). All fragmentation parameters were log-transformed prior to analysis.

The colonisation data from the experimental plots were also analysed with GLM to test differences in colonisation rate between species and effects of distance from the source population on colonisation rates.

Results

Natural distribution pattern

In total 85 patches of *S. latifolia* were found with a maximum number of plants ranging from 1 to over 13.000 and with different degrees of isolation (Fig. 4.3). Patch size was not correlated with any isolation parameter (all $P>0.05$). Eight patches consisted of only 1 or 2 male plants, unsuitable for the larval stage of the herbivore. Of the remaining patches only 2 did not show any signs of herbivory by *H. bicruris*. Both were not extremely isolated, but rather small (Fig. 4.3). In 66 patches, we were able to collect caterpillars. As was expected, the number of caterpillars collected during the 3 years depended strongly on the patch size (max. number of plants) (Fig. 4.4). The number of caterpillars collected was independent of any isolation parameter (Anova's, all $P>0.05$).

Based on collected caterpillars that were parasitised and visual observations (in patches where caterpillars could not be collected an adult *M. tristis* was observed once) we could detect the presence of *M. tristis, E. tristis and B. variator* in 53, 31 and 40 patches, respectively (Fig. 4.3). In total these parasitoids were found in 22.9, 11.7 and 4.9% of the 1773 caterpillars that were collected during the 3 years, respectively.

Whether the presence of *M. tristis* was observed or not was related to the number of caterpillars collected in a patch (Table 4.1). The probability of observing *M. tristis* increased with the number of plants in a patch (Table 4.1), with a 50% probability of observation in patches consisting of 5 plants (Fig. 4.5 A). The probability to observe *M. tristis* was not related to any of the nearest neighbour estimates (Table 4.1), but for very small values of alpha the presence of *M. tristis* was significantly affected by connectivity (S_{plant} and S_{spec}) in a model that also contained patch size (Fig. 4.6 A).

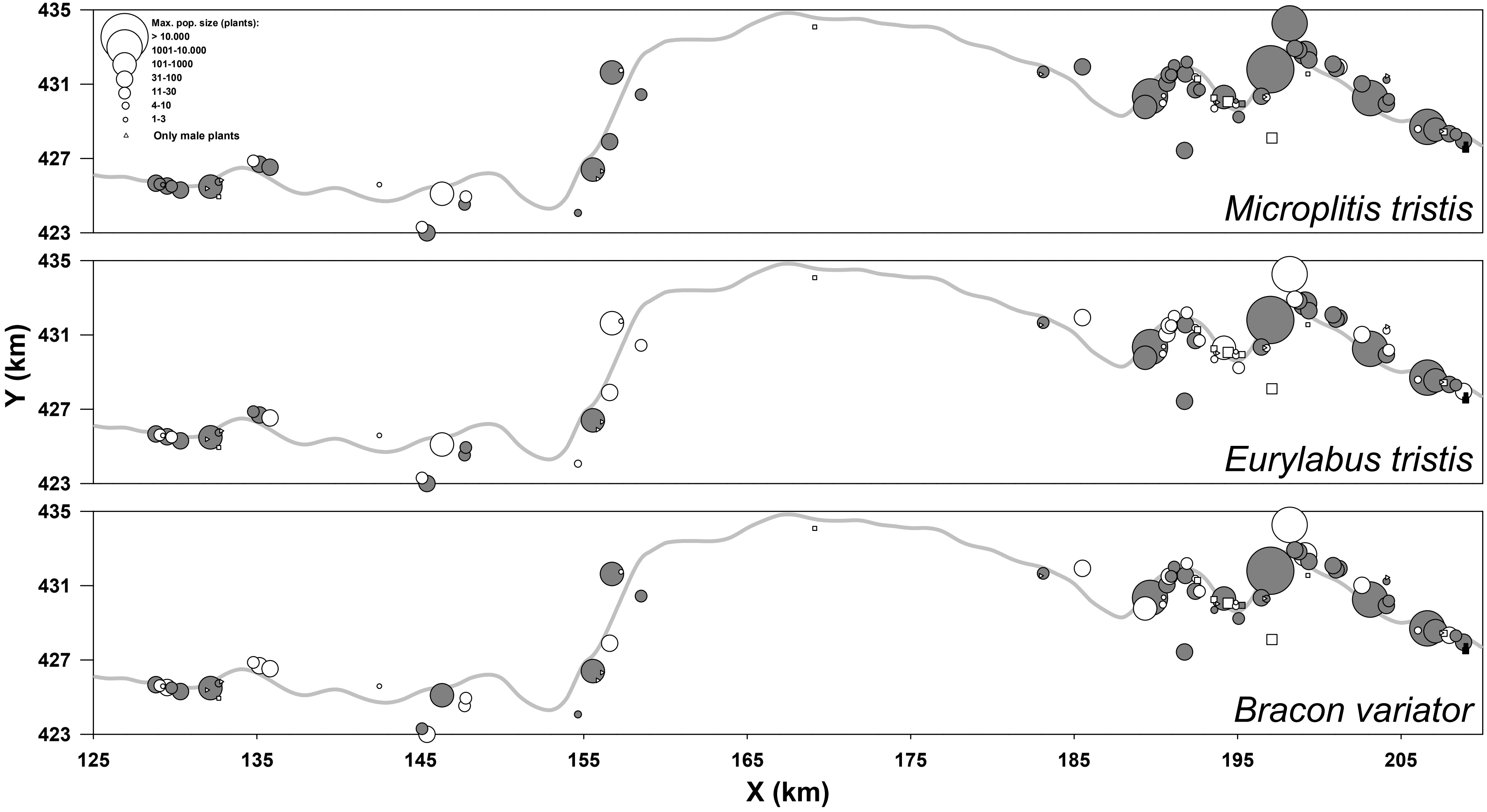


Figure 4.3 Maps showing the distribution patterns of the herbivore *H. bicruris* and its parasitoids *M. tristis* (top), *E. tristis* (centre) and *B. variator* (bottom panel) along the river. In all three maps the size of natural plant patches of *S. latifolia* is shown by the size of the symbol (only shown for circles). It is indicated whether in the plant patches no herbivory has been observed (black squares), herbivory was observed but no caterpillars were collected (open squares), caterpillars were collected but the parasitoid was not found (open circles), or caterpillars were collected and the parasitoid was observed (grey circles).

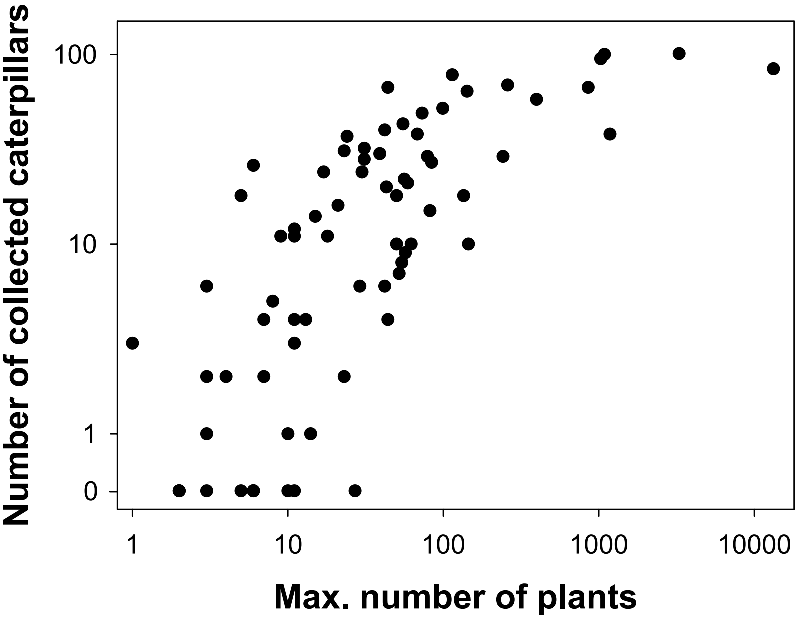


Figure 4.4 Relationship between the maximum number of *S. latifolia* plants and the number of collected caterpillars of *H. bicruris* in natural plant patches.

For *B. variator* similar results were found as for *M. tristis.* The probability of observing *B. variator* increased with the number of collected caterpillars and/or the number of plants (Table 4.3), although less strongly than for *M. tristis,* as can be observed from the wider range between the 10 % and 90 % probability of observation (Fig. 4.5 C). Again, very small values of alpha led to a significant effect of connectivity in combination with plant number on the presence of *B. variator* (Fig. 4.6 C), whereas other isolation estimates did not have any effects (Table 4.3).

The dispersal pattern of *E. tristis* seems different from the other two parasitoids. Its presence was highly significantly explained by size of the patch and/or the number of caterpillars, with only a few observations in patches of less than 50 plants (Fig. 4.5 B, Table 4.2). Additionally, several isolation estimates could explain the observations (Table 4.2). All nearest neighbour estimates were significantly related to the presence of *E. tristis* in all tested models (Fig. 4.5 B, Table 4.2). Presence of *E. tristis* significantly increased with connectivity for high values of alpha, but only if we used the non-species specific connectivity measurement (S_{plant}). The best fit was obtained for values of alpha around 10 (Fig. 4.6 B).

Table 4.1 Coefficient estimates from logistic regression models explaining the observed presence of *M. tristis* in natural *S. latifolia* patches. Logistic models were composed of $C_1 + C_2$*factor A + C_3*factor B. Only significant coefficient estimates of C_2 and C_3 are shown (- =P>0.05, * =P<0.05, ** =P<0.01, *** =P<0.001). See method section for an explanation of the different parameters. $^\$$ S was tested with alpha ranging from 0.2-50 (yes if P<0.05 for a specific range of alpha values).

Factor A		Factor B							
		None	NNDfem	NND10	NNDmicro	S_{plant} for alpha range$^\$$		S_{spec} for alpha range$^\$$	
None	C_3		-	-	-	-		-	
Host number	C_2	2.67***	2.72***	2.61***	2.68***	yes	0.2-50	yes	0.2-50
	C_3		-	-	-	yes	0.2-0.6	yes	0.2-0.5
Plant number	C_2	2.09**	2.09**	2.02**	2.07**	yes	0.2-50	yes	0.2-50
	C_3		-	-	-	-		-	
None (offset hostnr.)	C_3		-	-	-	-		-	
Plant number (offset hostnr.)	C_2	1.58*	1.55*	1.48*	1.55*	yes	0.2-50	yes	0.2-50
	C_3		-	-	-	-		-	

Table 4.2 Coefficient estimates from logistic regression models explaining the observed presence of *E. tristis* in natural *S. latifolia* patches. Logistic models were composed of $C_1 + C_2$*factor A + C_3*factor B. Only significant coefficient estimates of C_2 and C_3 are shown (- =P>0.05, * =P<0.05, ** =P<0.01, *** =P<0.001). See method section for an explanation of the different parameters. $^\$$ S was tested with alpha ranging from 0.2-50 (yes if P<0.05 for a specific range of alpha values).

Factor A		Factor B							
		None	NNDfem	NND10	NNDeury	S_{plant} for alpha range$^\$$		S_{spec} for alpha range$^\$$	
None	C_3		-2.04**	-2.18**	-1.97**	yes	5-50	-	
Host number	C_2	2.59***	3.14***	3.37***	3.57***	yes	0.2-50	yes	0.2-50
	C_3		-2.61**	-2.76**	-2.33**	yes	2-50	-	0.2-0.5
Plant number	C_2	1.87***	1.97***	2.07***	2.08***	yes	0.2-50	yes	0.2-50
	C_3		-2.34*	-2.50**	-1.83**	yes	4.5-50	-	
None (offset hostnr.)	C_3		-2.06**	-2.11**	-1.60**	yes	4-50	-	
Plant number (offset hostnr.)	C_2	1.32*	1.42*	1.54*	1.55*	yes	0.2-50	yes	0.2-50
	C_3		-2.35**	-2.51**	-1.92**	yes	4-50	-	

Table 4.3 Coefficient estimates from logistic regression models explaining the observed presence of *B. variator* in natural *S. latifolia* patches. Logistic models were composed of $C_1 + C_2$*factor A + C_3*factor B. Only significant coefficient estimates of C_2 and C_3 are shown (- =$P>0.05$, * =$P<0.05$, ** =$P<0.01$, *** =$P<0.001$). See method section for an explanation of the different parameters. $^{\$}$ S was tested with alpha ranging from 0.2-50 (yes if $P<0.05$ for a specific range of alpha values).

Factor A		Factor B					
		None	NNDfem	NND10	NNDbrac	S_{plant} for alpha range$^{\$}$	S_{spec} for alpha range$^{\$}$
None	C_3		-	-	-	-	-
Host number	C_2	1.38**	1.38**	1.39**	1.49**	yes 0.2-50	yes 0.2-50
	C_3		-	-	-	yes 0.2-0.6	yes 0.2-0.5
Plant number	C_2	0.91*	0.95*	0.95*	0.88*	yes 0.2-50	yes 0.2-50
	C_3		-	-	-	-	-
None (offset hostnr.)	C_3		-	-	-	-	-
Plant number (offset hostnr.)	C_2	-	-	-	-	-	-
	C_3		-	-	-	-	-

Experiment

In most experimental plots only 10 or fewer plants actually flowered, but nevertheless colonisation by all species over the two years of study was observed. The herbivore, *H. bicruris*, colonised (observed as either eggs, herbivory, or caterpillars present) all but one of the 29 plots within one year (Figs. 4.7 and 4.8). The one exception might be due to the low number of female flowers in that plot. Despite this colonisation, in most plots only few caterpillars could be collected (mean=3.7±0.8 s.e.). In 13 plots only 1 or 2 caterpillars could be collected and in 4 plots no caterpillars were found.

The specialist gregarious *M. tristis* was observed in 15 out of the 28 plots that were suitable (colonisation by the herbivore was observed) (Figs. 4.7 and 4.8). On three occasions an adult *M. tristis* was observed in a patch where no parasitised hosts could be found. In one patch (Gen 2000) an adult female was observed even before any sign of the presence of the herbivore. Although the number of observed colonisations by *M. tristis* (Fig. 4. 8) was significantly lower than for *H. bicruris* (GLM, $P<0.001$), no relation was found between colonisation and distance from the nearest natural source (GLM, n.s.).

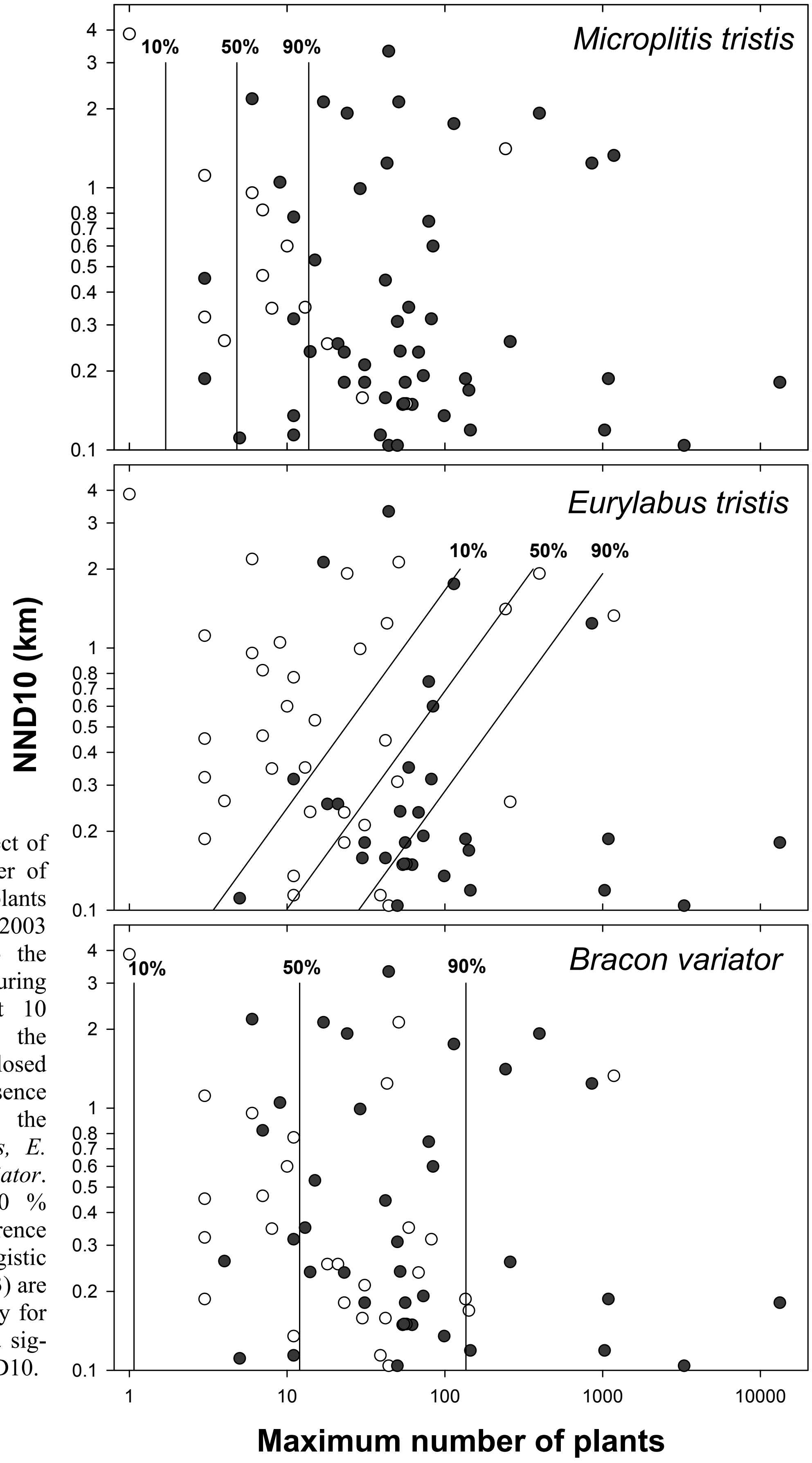
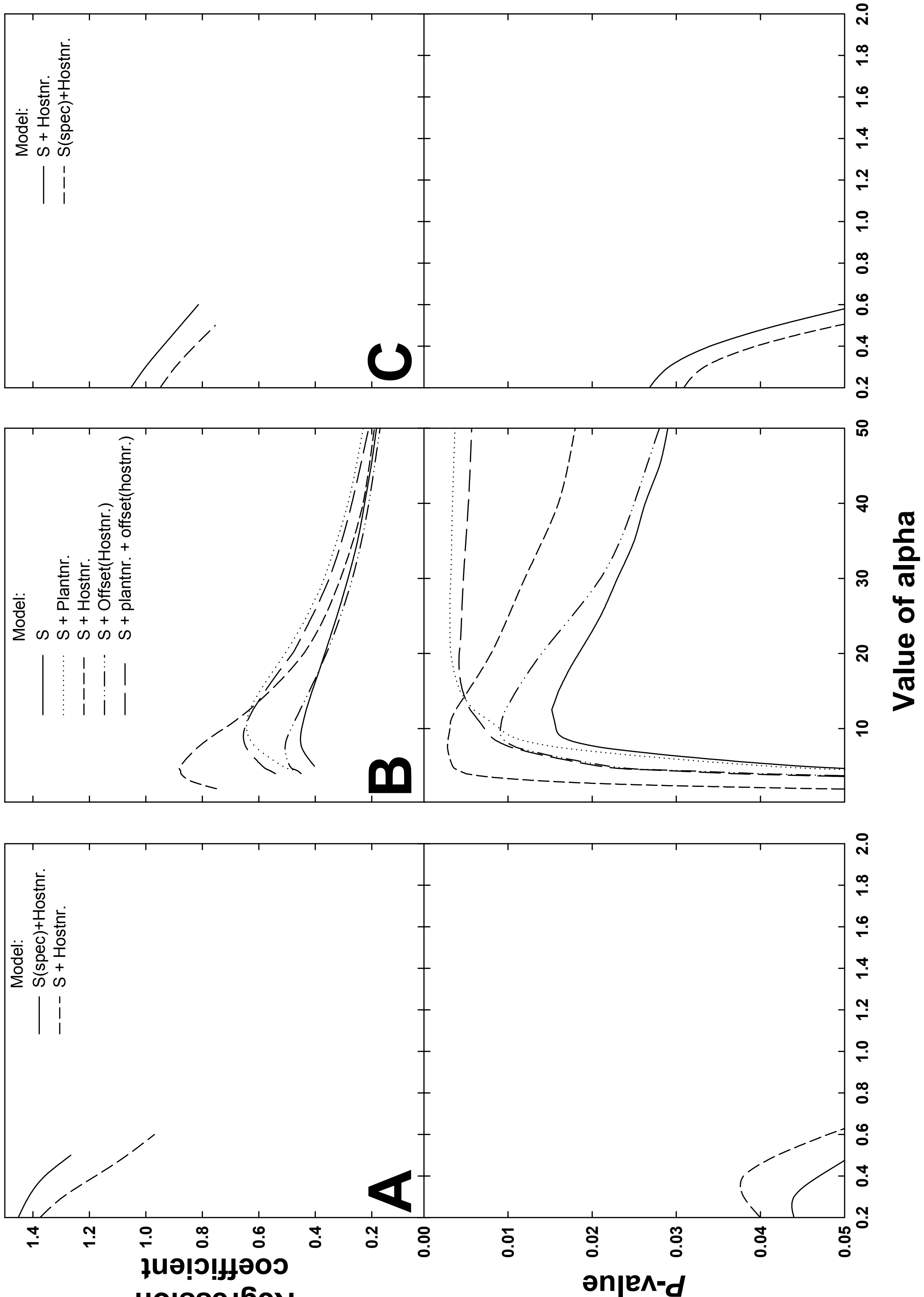


Figure 4.5 The effect of the maximum number of *S. latifolia* plants observed in 2001-2003 and the distance to the nearest neighbouring patch with at least 10 plants (NND10) on the observed presence (closed symbols) or absence (open symbols) of the parasitoids *M. tristis, E. tristis* and *B. variator*. The 10, 50 and 90 % probabilities of occurence as obtained from logistic models (Table 4.1-4.3) are shown. Note that only for *E. tristis* there was a significant effect of NND10.


A
Model:
S(spec)+Hostnr.
S + Hostnr.
B
Model:
S
S + Plantnr.
S + Hostnr.
S + Offset(Hostnr.)
S + plantnr. + offset(hostnr.)
C
Model:
S + Hostnr.
S(spec)+Hostnr.
Regression coefficient
0.2
0.4
0.6
0.8
1.0
1.2
1.4
P-value
0.00
0.01
0.02
0.03
0.04
0.05
Value of alpha
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
10
20
30
40
50

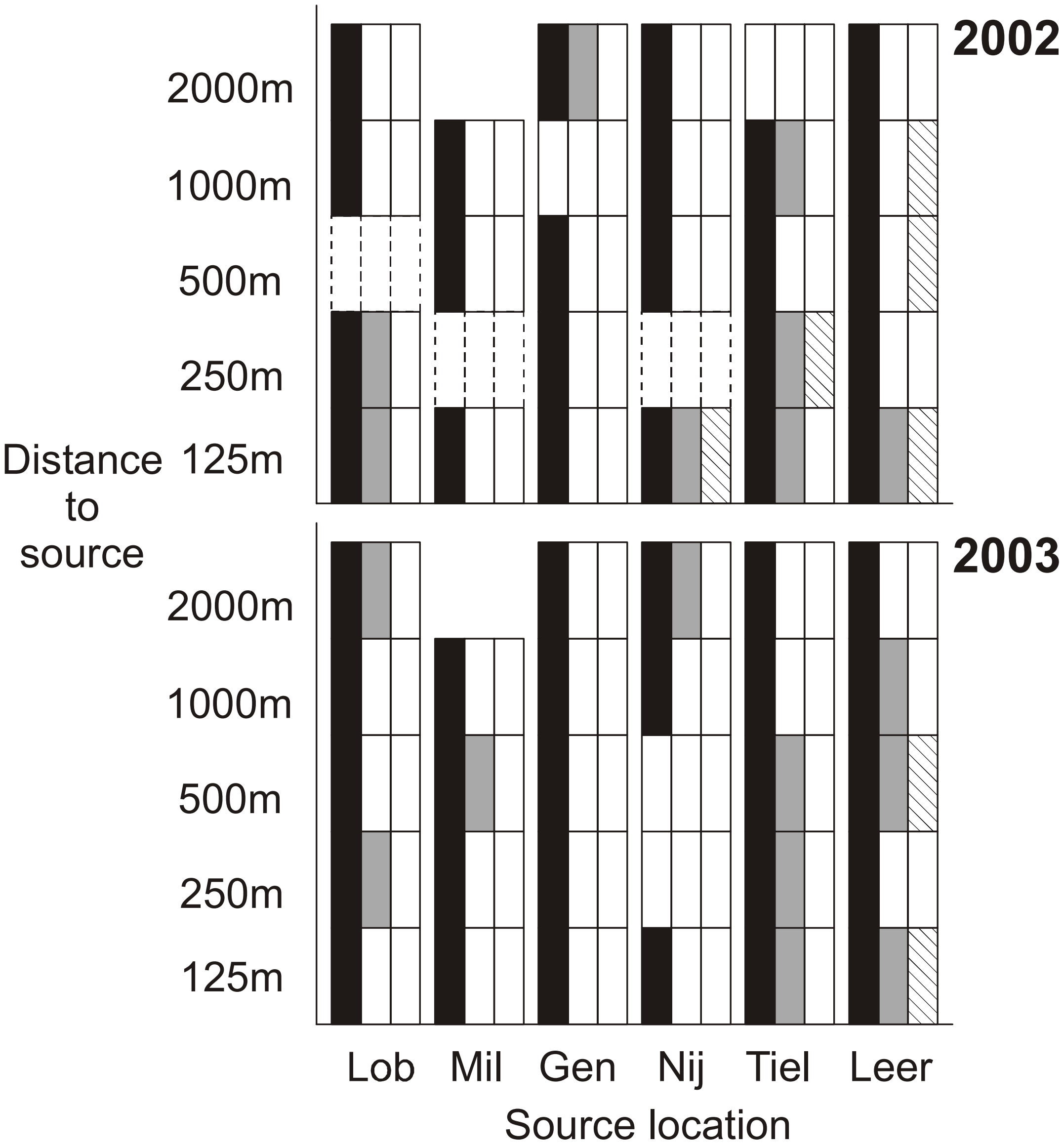


Figure 4.7 Observations of colonisations for the herbivore *H. bicruris* (black) and its parasitoids *M. tristis* (grey) and *B. variator* (hatched) in experimental patches of *S. latifolia* in 2002 (top) and 2003 (bottom). Dashed lines indicate that no flowering of the plants was observed, making patches unsuitable for the herbivore. Names of the sources correspond with Fig. 4.2.

Only one plot was colonised by the specialist parasitoid *E. tristis* (Lob1000) and only 5 out of the 28 suitable patches were colonised by the generalist *B. variator* (Figs. 4.7 and 4.8). From the colonisation data it is clear that, within the study period of two years, all species could reach a 1 km distant patch and that *M. tristis* was observed even in 3 out of the 5 patches at 2 km distance.

Figure 4.6 (opposite page) Alpha ranges for which different models resulted in a significant effect of connectivity (S) on the presence of the different parasitoid species in natural *S. latifolia* patches. The effects of different alpha values on the regression coefficient and the significance level (*P*-value) of the connectivity effect are shown for the parasitoids *M. tristis* (A), *E. tristis* (B) and *B. variator* (C).

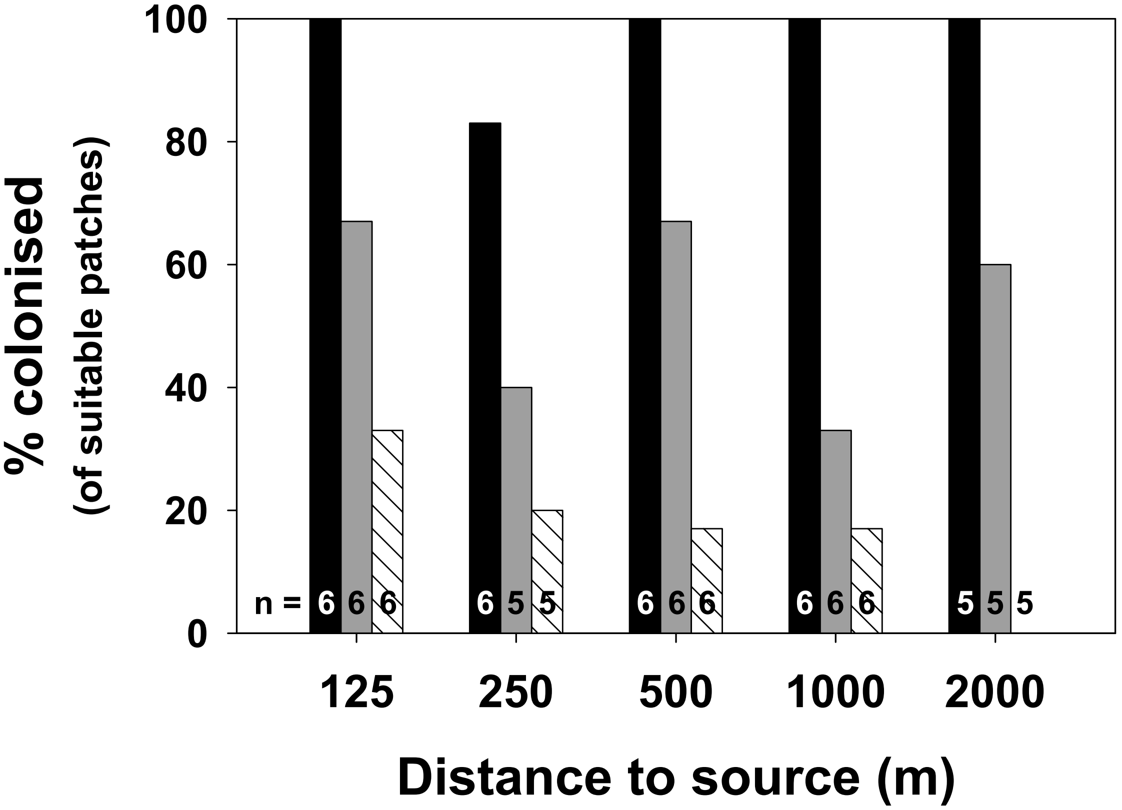


Figure 4.8 Percentage of experimental patches of *S. latifolia* located at different distances from natural source populations, that are colonised by the herbivore *H. bicruris* (black) and its parasitoids *M. tristis* (grey) and *B. variator* (hatched). Combined observations of 2002 and 2003.

Discussion

The natural distribution patterns

It is clear that the presence of the herbivore, *H. bicruris*, was not negatively affected by the current level of fragmentation, as it was observed in practically all natural *S. latifolia* patches.

The natural distribution patterns of the parasitoids indicate that the different species attacking *H. bicruris* are differently affected by habitat fragmentation. In fact, only one parasitoid, the solitary specialist *E. tristis*, showed a dispersal pattern that was related to the degree of isolation of plant patches. For this species a lower occurrence was observed in patches that were more isolated based on both nearest neighbour estimates and on connectivity values. The dispersal patterns of the two gregarious parasitoids, the specialist *M. tristis* and the generalist *B. variator* were not related to isolation at the scale of this study. For all three parasitoids, however, a small patch size measured as either plant number or host number resulted in a decrease in the probability of occurrence. This effect was strongest for *E. tristis* where parasitoids were almost completely absent from patches where less than 50 plants were present, whereas *B. variator* and especially *M. tristis*, were regularly observed in patches consisting of 10 plants or less.

The value of alpha (the strength of the effect of distance on connectivity) for which the generalised linear models, with a connectivity estimate incorporated, had the lowest *P*-value and the largest regression coefficient, was around 10 for *E. tristis*. At this value the connectivity measures are highly correlated with the nearest neighbour distances. Apparently with such high values of alpha the distance to the nearest patch(es) has a larger impact on the presence of the parasitoid than the size of the surrounding patches. This might explain why all nearest neighbour estimates could explain the observations well. Interestingly, incorporating the presence or absence of herbivore and parasitoid species in neighbouring patches (p_j) in the connectivity estimates could not improve the models, but rather decreased their predictive power in the case of *E. tristis*. If the number of dispersers (and colonisers) of *E. tristis* is indeed diluted with distance by a negative exponential, as assumed in the incidence function model (Hanski 1994), the average dispersal distance is 1/alpha (Moilanen and Nieminen 2002). This would mean a typical dispersion distance for *E. tristis* of about 100 m. Similarly, *M. tristis* and *B. variator* which show some correlations with values of alpha close to 0.2, would have a typical dispersal distance of more than 5 km, a distance much larger than most of the distances between patches in our study area.

The effect of plant patch size on the presence of the different parasitoid species is apparent for all species. However, the chance of finding parasitoids depends on the number of caterpillars collected, and in small patches few caterpillars are available so effects of patch size cannot be distinguished from effects of sample size. Incorporating an offset in the model for the number of collected hosts indicates whether plant number in a patch has an effect over and above sample size, but the two are still confounded. In fact, the number of small patches occupied by a parasitoid might be underestimated due to small host sample sizes. For *M. tristis* and *B. variator* a higher incidence would strengthen the conclusion that their presence is not strongly affected by fragmentation. For *E. tristis,* the conclusions might change but the very small incidence of the parasitoid in the experimental plots suggests that small populations are indeed unlikely to be colonised. Furthermore, it would not affect conclusions regarding isolation because the number of hosts collected is not correlated with isolation measurements. In many other studies reduced presence of species in small patches due to increased levels of extinction and decreased levels of (re)colonisation has been found for both hosts and parasitoids (e.g. Dempster *et al.* 1995a, Förare and Solbreck 1997).

The colonisation experiment

Whereas the presences of herbivores and parasitoids in the natural patches may be affected by local population dynamics, extinctions, and the age of the patches, the colonisation experiment can give more information on the actual dispersal abilities of the species. In fact, the results of the colonisation experiment correspond well with the results obtained from the natural distribution patterns. Similar to the natural patches, the herbivore, *H. bicruris,* had colonised virtually all experimental plots, independent of the distance from a potential source population, within the first year. This indicates a strong dispersal capacity and a good plant patch locating ability for this moth because even the smallest patches of only one plant were inhabited. Presumably the moth, that is a nectarfeeder, pollinator and herbivore on *S. latifolia,* is attracted at long distances by the strong odour that the flowers produce at dusk and during the night (Dötterl 2004). A study in a wind tunnel showed that both male and female *H. bicruris* moths strongly responded to odour emitting *S. latifolia* flowers (Brantjes 1981). From many other moths it is known that odour sources, especially pheromone sources can be located from several kilometres distance (see Cardé and Millar 2003). Furthermore, noctuid moths of similar size and shape have been shown to be strong fliers capable of dispersing hundreds of kilometers with averages even in the order of tens of kilometres, although most studies are performed on males of agricultural important species (Förare and Solbreck 1997, Schneider 1999).

M. tristis was also very efficient in colonising the experimental plots. In more than half of the plots, colonisation by *M. tristis* was observed within 1.5 years, including plots at 2000 meters from the nearest source population. *B. variator* colonised only a few experimental plots, and *E. tristis* was observed in only a single plot. Analysis of the natural dispersal patterns already showed that the probablity of occurrence for *E. tristis* and *B. variator* was lower in small patches, which might explain why colonisation by these species was not observed more frequently in the experimental plots that often consisted of less than 10 flowering plants.

The natural distribution pattern and the results from the colonisation experiment both show that the herbivore, *H. bicruris* is an extremely good disperser. The parasitoids differ in their dispersal abilities, the specialist solitary *E. tristis* having the worst dispersal and colonising ability. The gregarious specialist *M. tristis* is a good disperser, probably similar to the gregarious generalist *B. variator*, but the second is probably also responsive to alternative hosts in the surrounding environment.

Differences in dispersal abilities between parasitoid species

Our results are in agreement with those of several other studies showing that different species of parasitoids using the same herbivore host species can respond differently to the spatial pattern of the habitat (Brodmann *et al.* 1997, Roland and Taylor 1997, Doak 2000, van Nouhuys and Hanski 2002). Several factors underlying distribution patterns of species might explain why different species of parasitoids react differently to fragmentation, even when they use the same herbivore host species.

First of all, the number of dispersers (and thus potential colonisers) may be different due to the differences in total population sizes. In a study on colonisation of fragmented clover habitats, Krues and Tscharntke (1994) observed that the number of colonised patches corresponded with the number of individuals of particular species in the source habitat (for both herbivores and parasitoids). This might well explain why the solitary specialist *E. tristis* seems to be more affected by fragmentation than the other parasitoids. Although the parasitism rate of *E. tristis* is only 2.4 times smaller than for the gregarious *M. tristis*, the latter produces on average 18 individuals per parasitised host, leading to a potentially 45 times higher number of progeny, i.e. potential dispersers. When we consider that the sex ratio of *M. tristis* is female biased and that of *E. tristis* is 0.5 (Chapter 2) the difference might even be larger. In addition, small population sizes of *E. tristis* might leave them more prone to local extinctions (van Nouhuys and Tay 2001).

A second factor that is often suggested to explain differences in distribution patterns among species in fragmented landscapes, is the level of resource specialisation (Thomas *et al.* 1992, Zabel and Tscharntke 1998). Highly generalist parasitoid species are not expected to show strong effects of isolation of the habitat patches of the host if alternative hosts are available in the surrounding matrix habitat. This might explain why we did not find any effects of isolation in *B. variator* in our study area, though we do not know to what extent alternative hosts are present in the matrix habitat. If only certain habitat types contain alternative hosts it might explain why most colonisations by *B. variator* in the experimental plots appeared near the same source (Leer, Fig. 4.7). Alternative hosts for *B. variator* might also lead to a larger overall population size and weaken the relationship between its occurrence and the population sizes of *S. latifolia* and *H. bicruris,* as we observe. Studies where a distinction has been made between specialist and generalist parasitoids and predators have indeed reported that in general specialist species are more vulnerable to isolation than generalists (Kruess and Tscharntke 2000), but whether this is due to a larger number of dispersers in the source habitat or due to the availability of alternative hosts in the isolated habitats or the matrix or both is unknown.

Thirdly, differences in the distribution pattern between specialist parasitoids can also be explained by the differences in dispersal behaviour of parasitoid species (Brodmann *et al.* 1997, van Nouhuys and Hanski 2002). Unfortunately not much is known about dispersal behaviour of parasitoids between habitat patches, but we can speculate about the role of several parasitoid characteristics in causing differences between parasitoid species in their dispersal abilities in a fragmented landscape.

The first characteristic is dispersal capacity. It might be expected that bigger parasitoids are stronger fliers and thus disperse better (Tscharntke and Brandl 2004). This has been shown for a small parasitoid species at a scale of 20 m. (Ellers *et al.* 1998). Also van Nouhuys and Hanski (2002) show that the largest of two parasitoid species has a better dispersal ability and Roland and Taylor (1997) show that the spatial scale at which parasitism rate responds to forest structure is positively correlated with the relative body size of four tachinid parasitoids. This study however, shows the opposite where the smaller parasitoid species are less affected by isolation than the large *E. tristis*. Also other studies have shown that even very small egg-parasitoids do regularly cross at least 1 km of water (Antolin and Strong 1987). Apparently, active dispersal for which large sizes or wingspan seem to fit, is not always the most important way of parasitoid dispersal. Studies performed in the first half of the 20th century report many small Hymenopterans in air-samples taken at tens to thousands of meters by airplanes and balloons (Glick 1939, Freeman 1945), suggest that passive dispersal is a common phenomenon for small parasitoids. Studies on plant seeds show that light seeds, once they are uplifted several meters, can be transported many kilometres (Tackenberg 2003). This may explain the good dispersal ability of the small *M. tristis*, as well as the observed dispersal ability of small egg-parasitoids by Antolin and Strong (1987).

However, without an active directional mode of dispersal the chances of reaching a suitable habitat patch become very small at long distances. Desouhant *et al.* (2003) observed that the directions of dispersal by the parasitoid *Venturia canescens* within 100 m could be divided in up-wind and downwind, suggesting that some species use both modes of dispersal. This has also been suggested as the normal dispersal mechanism for fig wasps (Compton *et al.* 2000) and other flying insect species (Stein *et al.* 1994). Not only size is important, but also the lifespan of a parasitoid species might determine how far it is able to disperse, but this has not yet been investigated.

Realised dispersal is not only a function of the dispersal capacity but also of the tendency to leave a patch in the first place, although they might be evolutionary linked. This second characteristic might be related to local host availability. When host availability is generally low we might expect a parasitoid to have a high innate tendency to leave host patches and migrate to other patches. Perhaps, parasitoids specialised on solitary hosts with low local abundances, as is the case with *H. bicruris*, have higher dispersal tendencies compared with specialists on aggregated and abundant hosts (Fournier and Boivin 2000).

Gregariousness of parasitoid species may also be linked to dispersal tendency as it will generally decrease local host availability by intraspecific competition. *M. tristis* is a highly gregarious parasitoid that produces on average 18 female biased offspring in one host (Chapter 2). This would mean that in small patches with a limited number of hosts available, strong intraspecific competition between females could occur. Natural selection for females having a high dispersal tendency and ability might have occurred, to avoid competition and detrimental superparasitism of hosts. Studies have shown that frequency dependent dispersal exists for parasitoids, suggesting that competition is likely to affect dispersal tendency (French and Travis 2001). Although van Nouhuys and Hanski (2002) found that a solitary species was a better disperser when compared with a gregarious species, its host availability at any given time is very limited due to the short time for which a host is suitable, explaining why this parasitoid might have evolved a high dispersal ability (van Nouhuys and Ehrnsten 2004).

Differences in distribution patterns among parasitoid species might also be caused by differences in chances of successful establishment after colonising a patch or differences in local extinction rate. For a parasitoid to establish a new population it is important that a newly arrived female can produce both male and female offspring. Also, a large number of offspring can limit the probability of local extinction. Gregarious species, such as *M. tristis*, might have a high probability of successful establishment and persistence in a patch, because they can produce many offspring, even when few hosts are parasitised, as compared to the solitary *E. tristis.* Our results are in contrast with the idea that the worst competitor should be the best disperser to persist in the system (van Nouhuys and Hanski 2002). In this study, the worst disperser, *E. tristis,* is probably also the worst competitor because it can only develop in caterpillars that are not parasitised by others. In several dissected caterpillars from which larvae of *M. tristis* had emerged, a small larva of *E. tristis* was discovered (Chapter 2).

Effect of fragmentation on the plant-host-parasitoid system

Although the herbivore *H. bicruris* is a strong disperser, it does not escape long from the most abundant parasitoid in the study area, *M. tristis*, even in isolated patches. *M. tristis* seems to be a relatively good disperser, at least up to distances of a few kilometres. Only the specialist *E. tristis* seems to be affected in its ability to (re)colonise patches by the current level of fragmentation in the system. The generalist *B. variator* might be present in the surrounding habitats, independent on the level of fragmentation of *S. latifolia* patches.

Therefore it appears that fragmentation has currently little effect on parasitism level. Only the absence of *E. tristis* might affect the local population dynamics in the system but that depends on the effect it has on the local herbivore population. If the moth can immigrate very quickly from other populations, the effects might be small. A more detailed study on the effects of habitat fragmentation on parasitism rate and herbivory level in the system will be presented elsewhere (Chapter 5).

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Chapter 5

Effects of habitat fragmentation on herbivory and parasitism rate of the plant *Silene latifolia* and its specialist herbivore *Hadena bicruris*

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Abstract

Habitat fragmentation may 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* patches to examine the effects of patch size and isolation on the level of herbivory by the seed predating noctuid *Hadena bicruris* and the rate of parasitism of the herbivore by its parasitoids. Furthermore, we measured oviposition rate, herbivory and parasitism in differently sized experimental patches and examined whether edge effects did occur.

In the natural patches, the level of herbivory increased with fragmentation, both with declining patch size and increasing degree of isolation. Parasitism rate and the number of parasitoid species declined with decreasing patch size.

Oviposition rate and herbivory level, but not parasitism rate, decreased with patch size in the experimental patches, suggesting a behavioural response in oviposition rate of the moth. Oviposition rate and herbivory, but not parasitism rate, were increasing near the edges of patches.

Our research 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 patches with reduced patch size, increased isolation and a higher edge:interior ratio. Although the rate of parasitism and the number of parasitoid species declined with decreasing patch size in the natural patches, we argue that in this system it is unlikely that this decline made a major contribution to the increased herbivory.

Introduction

Major consequences of natural and human caused habitat fragmentation are a reduction of size, an increase in isolation, and an increase of the edge: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) and experience a large edge:interior ratio (Lehtinen *et al.* 2003). Furthermore, the increase in isolation can greatly reduce the exchange of genetic material through 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).

However, not only these direct effects of fragmentation may change population viability, also the changes in biotic interactions may affect 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 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 Tscharntke 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 patches (Zabel and Tscharntke 1998, Kruess and Tscharntke 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 Tscharntke 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, Tscharntke and Brandl 2004), leading to a reduction in parasitism in isolated and small habitats (Kruess and Tscharntke 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 individual insect larvae (Guillot and Vinson 1973, Powell 1989, Elzinga *et al.* 2003).

Not only the presence of animal species but also their individual behaviour may change in response to fragmentation. Several studies have shown that dispersal behaviour can be altered by patch 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 patches (Baguette *et al.* 2003). Differences in migration rate will obviously affect the number of individuals in a patch and therefore the level of herbivory or parasitism rate. Also oviposition rate of insects is affected by the size of plant patches (Maguire 1983, Shea *et al.* 2000), leading to differences in the level of herbivory.

Whereas the decrease in size and the increase in isolation are recognised as important factors leading to changes in herbivory level, the effects of changes in edge:interior ratio are less well studied (Fagan *et al.* 1999). Biotic interactions can be different in edges than in the centre of a patch, 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 patches usually have larger edge:interior ratios, these populations can be more strongly affected.

We have studied the effects of habitat fragmentation on the level of herbivory and the parasitism rate of the herbivore of the plant *S. latifolia* in the Netherlands. *S. latifolia* is subject to a very high level of pre-dispersal seed predation by the specialist noctuid herbivore *H. bicruris* that reduces the potential fitness of individual plants considerably by reducing the number of produced seeds (Biere and Honders 1996b, Wolfe 2002). In turn, the herbivore is attacked by several species of generalist and specialist parasitoids leading to an average parasitism rate of almost 50% (Chapter 2).

We measured the level of herbivory and the parasitism rate in many (semi-) natural *S. latifolia* patches differing in size as well as in the degree of isolation. Because the herbivore is a good disperser we do not expect differences in the level of herbivory that would be due to its absence (Chapter 4). However, we expect that the number of parasitoid species present in small and isolated patches is smaller, leading to a decrease in parasitism rate and an increase in herbivory.

Because the differences in herbivory and parasitism in the natural patches might also be a result of differences in biotic (e.g. plant density) and abiotic conditions (e.g. soil quality) of the location and not per se on the degree of fragmentation (Debinski and Holt 2000, Thomas *et al.* 2001), we additionally established experimental plant patches with a fixed plant density in an experimental garden, where both herbivores and parasitoids could colonise the plant patches. We measured oviposition rate, the level of herbivory of the plants and the rate of parasitism of the herbivores to study whether these parameters were affected by patch size. To indicate whether fragmentation effects could be due to edge-effects, we examined the differences between the centres and the edges of the large experimental patches.

Methods

Study system

Silene latifolia Poiret (= *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) predominantly on chalky sandy soil (Baker 1947). It is native to Eurasia, and has been introduced to North America where it is considered a pest species (Wolfe 2002).

S. latifolia 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* and *Autographa gamma* as most common visitors, although some hoverfly species may visit the flowers during the day (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 Europe the most important herbivore consuming the developing seeds is the specialist noctuid *H. bicruris* (Brantjes 1976b, Wirooks and Plassmann 1999, Wolfe 2002). *H. bicruris* Hufn. (Lepidoptera: Noctuidae), the Lychnis, is a night-active moth with a wingspan of 30-40 mm. Caterpillars can be found in the Netherlands from May until October (pers. obs.), 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 (L1) 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 is has consumed all the seeds, which usually occurs when the caterpillar has reached its fourth or fifth (hereafter L4 and L5, respectively) 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 (L4, L5) of *H. bicruris* that is most vulnerable to parasitoids. A detailed description of all the parasitoid species found in this study is presented elsewhere (Chapter 2).

The natural patches

We studied the level of herbivory and the level of parasitism of the herbivores by parasitoids in natural *S. latifolia* patches 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* (Chapter 4). The area consists of floodplains and river dikes with different types of management, soil and elevation. The floodplains are submersed usually in winter. While a large part is nature conservation area where only herds of wild horses and cattle graze, other parts are used for agriculture (mainly haymaking) or for industrial purposes (e.g. brick making factories). *S. latifolia* patches mainly grow on the elevated sandier parts but also in disturbed areas near industrial sites and along roadsides and field margins. Many patches of *S. latifolia* also grow on the river dikes, which are mostly mown twice a year. The surrounding area was unsuitable for *S. latifolia* due to heavy agriculture and less suitable soil. Due to these clear borders the area was very suitable to study effects of fragmentation as we could be sure that our fragmentation estimates were not strongly influenced by other populations.

We visited all populations several times a year in 2001, 2002 and 2003. From all populations we measured the size 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, we computed nearest neighbour distances between populations from coordinates of plants at the borders of the populations measured with a GPS system

(GIS*backpack*, Commetius, Leiderdorp, NL). Populations were distinguished if they were at least 100 m separated from each other.

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. Secondly, from at least 10 randomly selected female plants, or from all plants if 10 or less were present, we counted all undamaged seed capsules and capsules showing signs of herbivory. From 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 ºC 16/8h L/D to find out whether they were parasitised. 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 ºC 16/8 L/D prior to continued rearing. Caterpillars that died were dissected to determine whether they had been parasitised.

Experimental patches

To study effects of patch size on herbivory and parasitism rate we created experimental plant patches 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* has 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 patches separated 100 m from each other (the distance used to distinguish populations in the natural study area) in 2002 and in 2003 (Fig. 5.1). Small patches consisted of 16 plants arranged in a square (4 x 4) with 0.75 meters in between plants. Male and female plants were placed alternating in 2002 and randomly in 2003. The large patches were similarly arranged but consisted of 256 plants

(16 x 16 grid) creating groups of plants at 8 different distances from the edge (Fig. 4.1). In each year, plants were grown from seedlings in large pots (18 x 18 x 18 cm) with a mixture of clay, peat and sand mixture (resp. 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 patches was restricted to one quadrant (every week a different quadrant). In the remaining parts of the large patches 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 host had been collected to allow species to persist in the patches during the year.

Analyses

For natural patches, the level of herbivory was calculated both at the patch 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. We used a generalised linear model with a quasibinomial distribution (to account for overdispersion) and a logit link function (GLM) (McCullagh and Nelder 1989) to analyse whether herbivory was affected by patch size and/or nearest neighbour distance for each of the three years separately. As nearest neighbour distance we used both distance to the nearest *S. latifolia* patch and the nearest neighbour distance to a patch with at least 10 plants.

For analyses of parasitism rate in natural patches we combined data from all three years, as in many small patches we could collect only few or no caterpillars in every single year. We analysed effects of patch size (the maximum number of plants), nearest neighbour distance and the number of parasitoid species that were found (patches with no parasitised caterpillars were excluded from the analyses) on the proportion of parasitised caterpillars in a patch, using GLM. In addition, we tested whether nearest neighbour distance and patch size had an effect on the number of parasitoid species found in a natural patch (linear model).

For the experimental patches, we analysed effects of patch size and time (weekly counts) on the proportion of flowers with at least one egg (data 2002) with GLM. Three different measures were used: (i) size class (large or small), (ii) total number of flowers in the patch at the weekly census and (iii) total number of flowering plants at the weekly census. The level of herbivory and the parasitism rate (data 2003) were analysed similarly, but here we could only use the total numbers over the whole experimental period. In addition, effects of the position of plants relative to the edge of the patch on oviposition rate, herbivory level and parasitism rate were analysed with GLM for plants from the large patches.

All GLM analyses were performed in the freely available statistics software programme R (Venables *et al.* 2003) with log10 transformed variables.

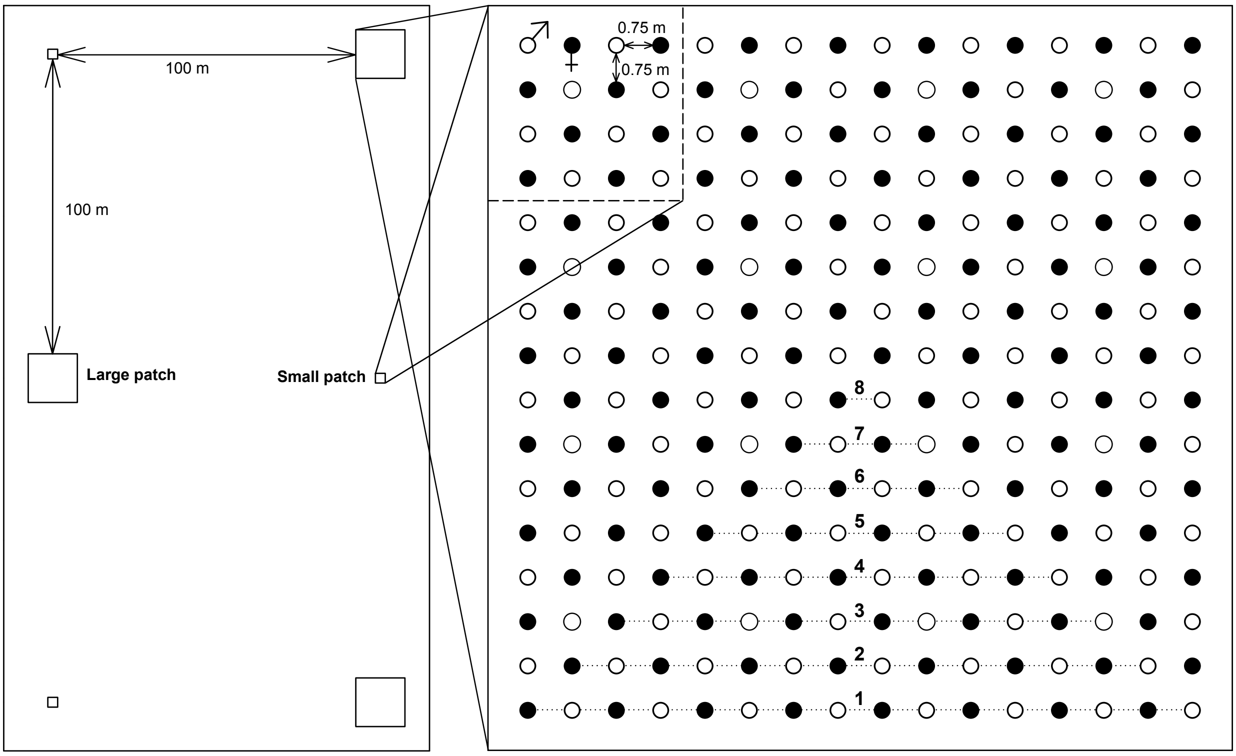

Figure 5.1 Experimental setup in 2002 of the small (16 plants) and large (256 plants) *S. latifolia* patches. In 2003 male and female plants were distributed randomly and large and small patches were transposed. Row numbers are indicated on one side of the large patch.

Results

Natural patches

In total we located 85 patches of *S. latifolia* in the study area ranging in size from 1 to 13.000 plants (median = 21 plants). Around 50 patches (median = 39 plants) each year were suitable to estimate the level of herbivory, the rest was disturbed by mowing, or contained only male plants. Each year, *H. bicruris* was present in almost every patch we visited, so almost no patches had zero herbivory (Fig. 5.2). 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 affected by *H. bicruris* (with at least one seed capsule showing signs of herbivory) was negatively correlated with the number of plants in a patch in 2001 and 2002 but in none of the years it was significantly correlated with nearest neighbour distances (Fig 5.2, Table 5.1 A). The proportion of seed capsules infested by *H. bicruris* decreased with increasing patch size in 2002 and 2003 (Fig. 5.3, Table 5.1 B). In 2001 and 2002 this proportion correlated positively with nearest neighbour distance (Fig. 5.3, Table 5.1 B). Using the nearest neighbour distance to a patch with at least 10 females instead, showed exactly the same patterns (data not shown).

Table 5.1 Anova tables for generalised linear models explaining (A) the proportion of *S. latifolia* plants damaged by *H. bicruris* (at least one seed capsule damaged) and (B) the proportion of seed capsules damaged, in natural plant patches. If the interaction is not significant, F values for the variables are derived from a model without interaction.

A

		2001 (error d.f.=46)		2002 (error d.f.=40)		2003 (error d.f.=49)	
Variables	d.f.	F-value	*P*-value	F-value	*P*-value	F-value	*P*-value
Patch size (number of plants)	1	5.00	0.030	16.44	<0.001	*2.08*	*0.156*
Isolation (nearest neighbour distance)	1	*0.12*	*0.731*	*2.96*	*0.093*	*0.011*	*0.917*
Interaction	1	*1.34*	*0.253*	*2.40*	*0.130*	*0.021*	*0.885*

B

		2001 (error d.f.=46)		2002 (error d.f.=40)		2003 (error d.f.=49)	
Variables	d.f.	F-value	*P*-value	F-value	*P*-value	F-value	*P*-value
Patch size (number of plants)	1	*2.86*	*0.097*	16.53	<0.001	5.00	0.030
Isolation (nearest neighbour distance)	1	7.86	0.007	*0.041*	*0.840*	*1.58*	*0.215*
Interaction	1	*0.40*	*0.530*	5.16	0.029	*0.67*	*0.417*

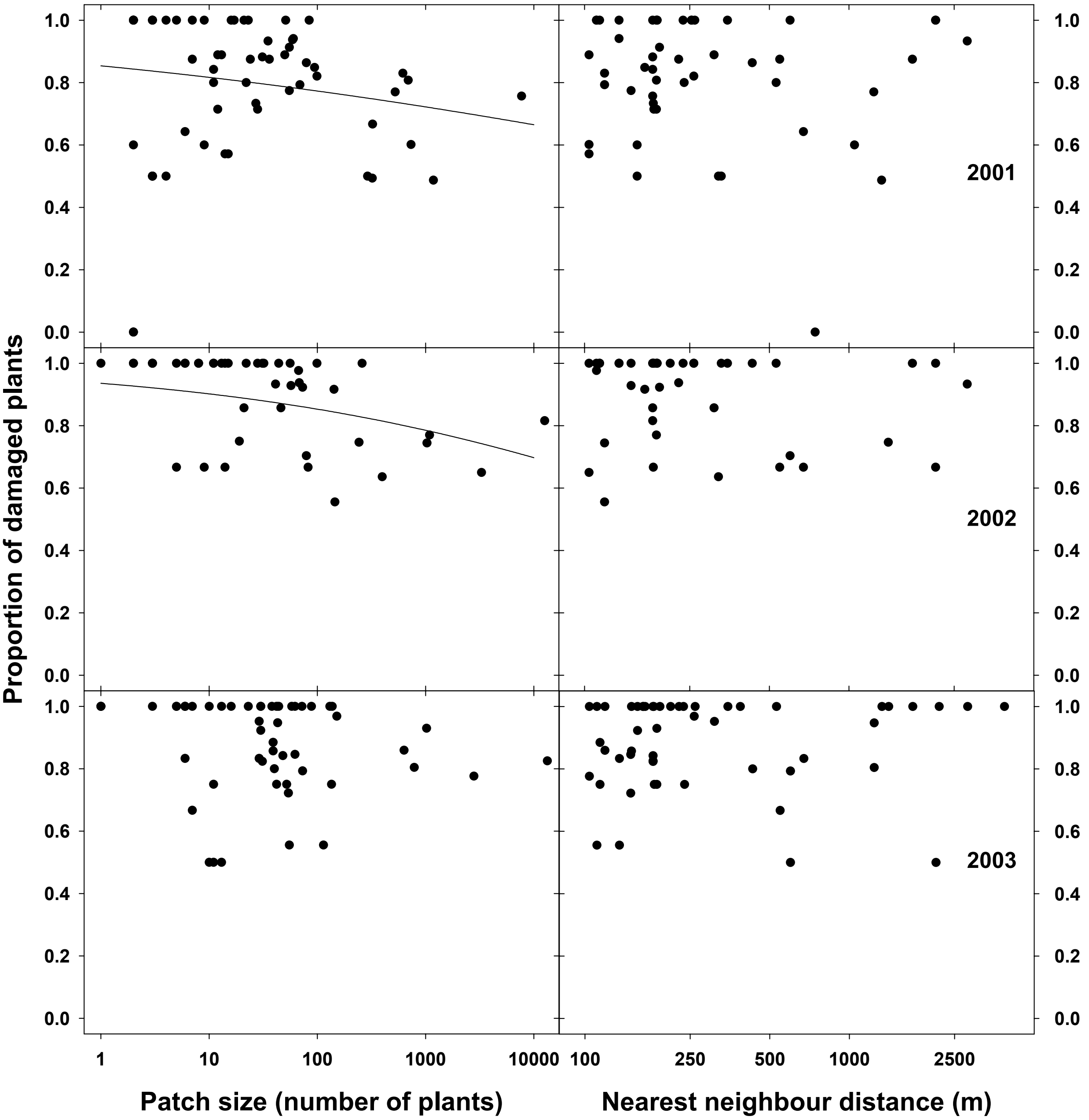

Figure 5.2 The proportion of *S. latifolia* plants damaged by *H. bicruris* (at least one seed capsule damaged) in natural patches in relation to patch size and isolation in 2001, 2002 and 2003. Lines show the results from a logistic regression with only the significant variables entered (see Table 5.1 A).

In about 80 % of all populations we found at least one caterpillar being parasitised. In total, at least 14 parasitoid species caused approximately a 50 % parasitism rate, of which 95 % was caused by four hymenopteran species: the small gregarious koinobiont *Microplitis tristis* (Braconidae), the large solitary larval-pupal parasitoid *Eurylabus tristis*, the small gregarious ectoparasitoid *Bracon variator* (Braconidae) and the large solitary koinobiont *Ophion pteridis* (Ichneumonidae). The parasitism rate of the caterpillars that we collected was positively correlated with the maximum number of

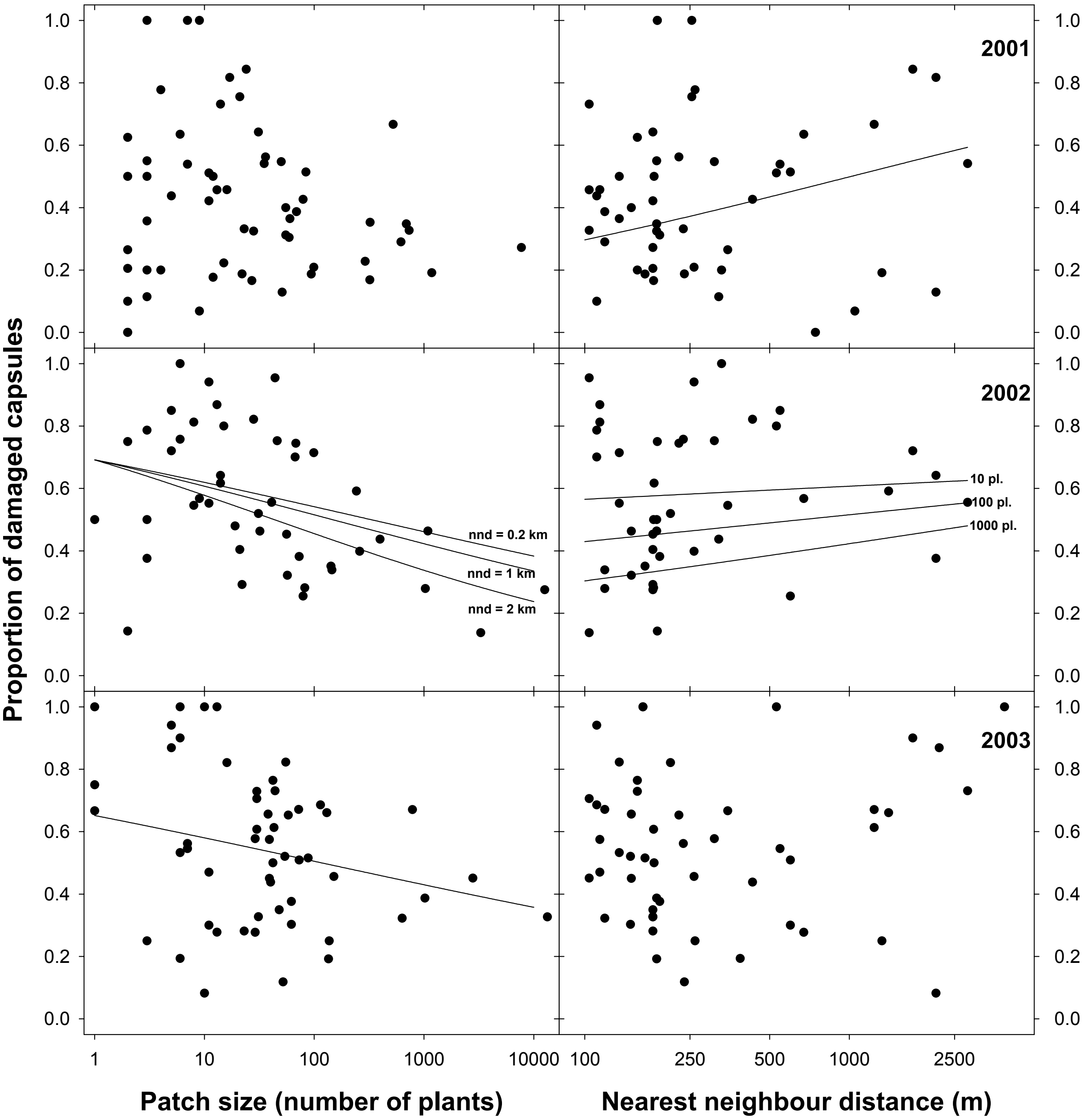

Figure 5.3 The proportion of seed capsules of *S. latifolia* damaged by *H. bicruris* in relation to patch size and isolation in natural plant patches in 2001, 2002 and 2003. Lines show the results of logistic regressions with only the significant variables and interactions added (see table 5.1 B). Interaction effects are shown by lines for different values of nearest neighbour distance (nnd) or patch size (number of plants (pl.)).

plants in the natural patch from which they originated, in the three years observed (Fig. 5.4 A, Table 5.2). Nearest neighbour distance only affected parasitism rate in interaction with patch size (Fig. 5.4 B, Table 5.2). Increasing nearest neighbour distance reduced parasitism rate in large but not in small patches. Using the nearest neighbour distance to a patch with at least 10 individual plants yielded similar results (data not shown). Parasitism was positively correlated with the number of parasitoid species we could find in a patch (Fig. 5.4 C, GLM, $F_{1,58}$ =12.67, P<0.001). However, comparing the resulting residual deviance with a model with patch size and the interaction with nearest neighbour distance, suggested that parasitoid species has less explanatory power (ANOVA, $F_{1,58}$ = 14.16, P<0.001). The number of parasitoid species that were found during the three years increased with an increase in patch size (linear regression, P<0.001, R^2 =0.24) but there was no relationship with nearest neighbour distance (Fig. 4.5).

Table 5.2 Anova table for a generalised linear model explaining the parasitism rate of *H. bicruris* in the natural patches over the three years (error d.f. = 56).

Variables	d.f.	F-value	*P*-value
Patch size (maximum number of plants)	1	22.65	<0.001
Isolation (nearest neighbour distance)	1	*1.40*	*0.242*
Interaction	1	8.74	0.005

Experimental patches

In 2002 we were able to check 6854 female flowers for eggs in the patches in 12 weeks from May 24 until August 9 after which flower production by the plants rapidly ceased. In all patches more than 95 % of the plants flowered during the experiment. In 27 % of all the flowers the herbivore had deposited an egg. The average number of eggs deposited per plant changed greatly during the year (Fig 5.6). The oviposition rate on average decreased with patch size, both when patch size was quantified as the total number of plants (Fig. 5.7 A, Table 5.3 A), number of flowers (Table 5.3 B) or number of flowering plants (Table 5.3 C). Also the average number of eggs that was deposited in flowers where already one or more eggs were present was higher in smaller patches but the difference between patch sizes was not statistically significant ($F_{1,4}$ =2.82, P=0.17, Fig. 5.7 B).

The proportion of seed capsules destroyed by *H. bicruris* in the experiment in 2003 was on average larger in smaller patches, but the difference between the two patch sizes was not statistically significant ($F_{1,4}$ =3.26, P=0.15, Fig. 5.7 C). Only for the proportion of secondary predated capsules patch size had a marginally significant effect ($F_{1,4}$ =6.21, P=0.07, Fig. 5.7 C). However, because total parasitism was not different between the two patch sizes (F<0.001, P=0.99, Fig 5.7 D), it is unclear why caterpillars in large patches would consume 2-3 seed capsules after leaving the primary seed capsule and caterpillars

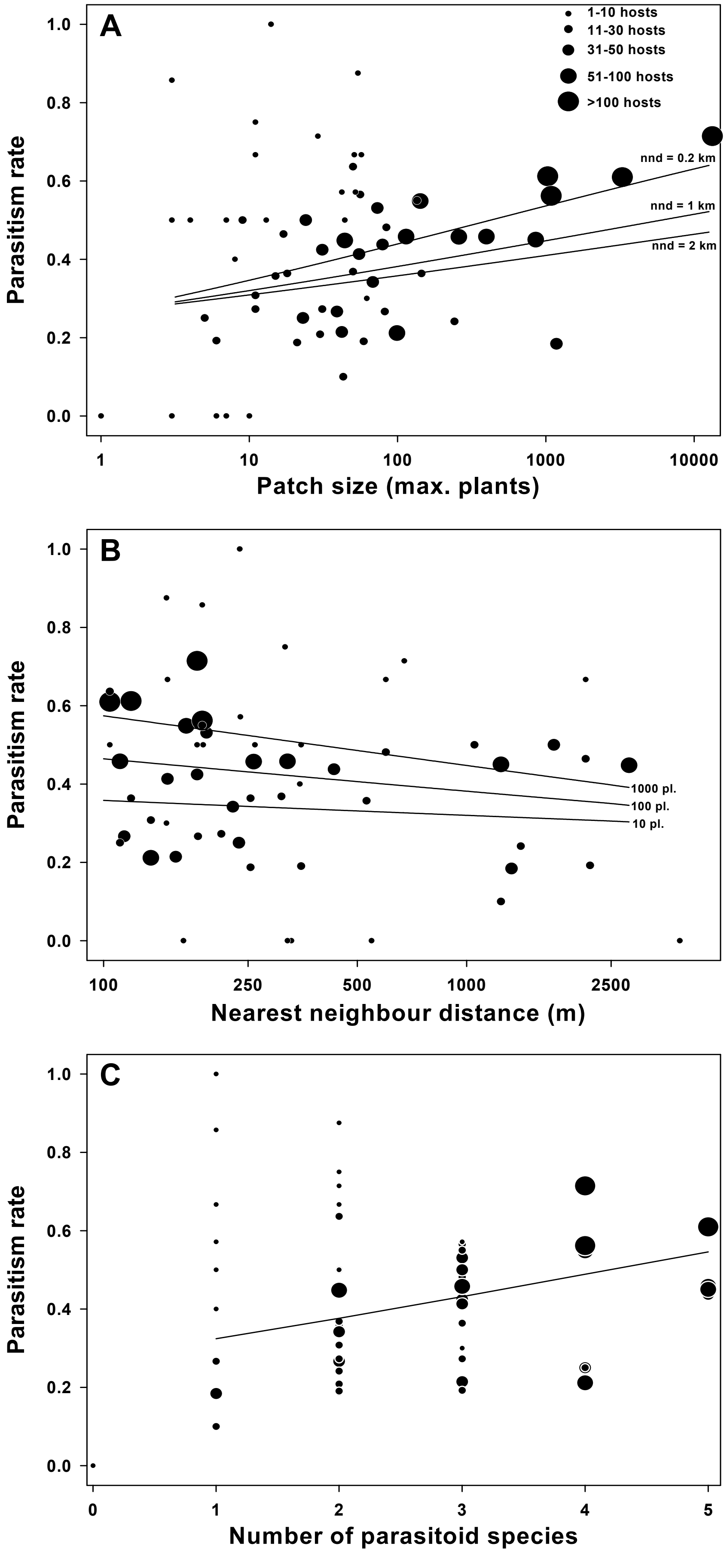

Figure 5.4 The parasitism rate of *H. bicruris* caterpillars in natural *S. latifolia* patches over three years as a function of (A) patch size, (B) isolation and (C) the number of observed parasitoid species. The size of the dots indicates the sample size of caterpillars (hosts) on which the datapoint is based. Lines show results from a logistic regression with only significant variables entered (see Table 5.3 and text). Interaction effects are shown by lines for different values of nearest neighbour distance (nnd) or patch size (number of plants (pl.)).

in small patches 3-4. One possibility is pollen limitation in small patches. This could lead to seed capsules with less seeds and nutritional value, forcing the caterpillars to consume more but smaller seed capsules. However, we think the reason is rather that in small patches several primary capsules were used as secondary seed capsules. On several occasions we observed that the cannibalistic large caterpillars (Elzinga *et al.* 2002) consumed the contents of a primary capsule including its original inhabitant when they encountered it.

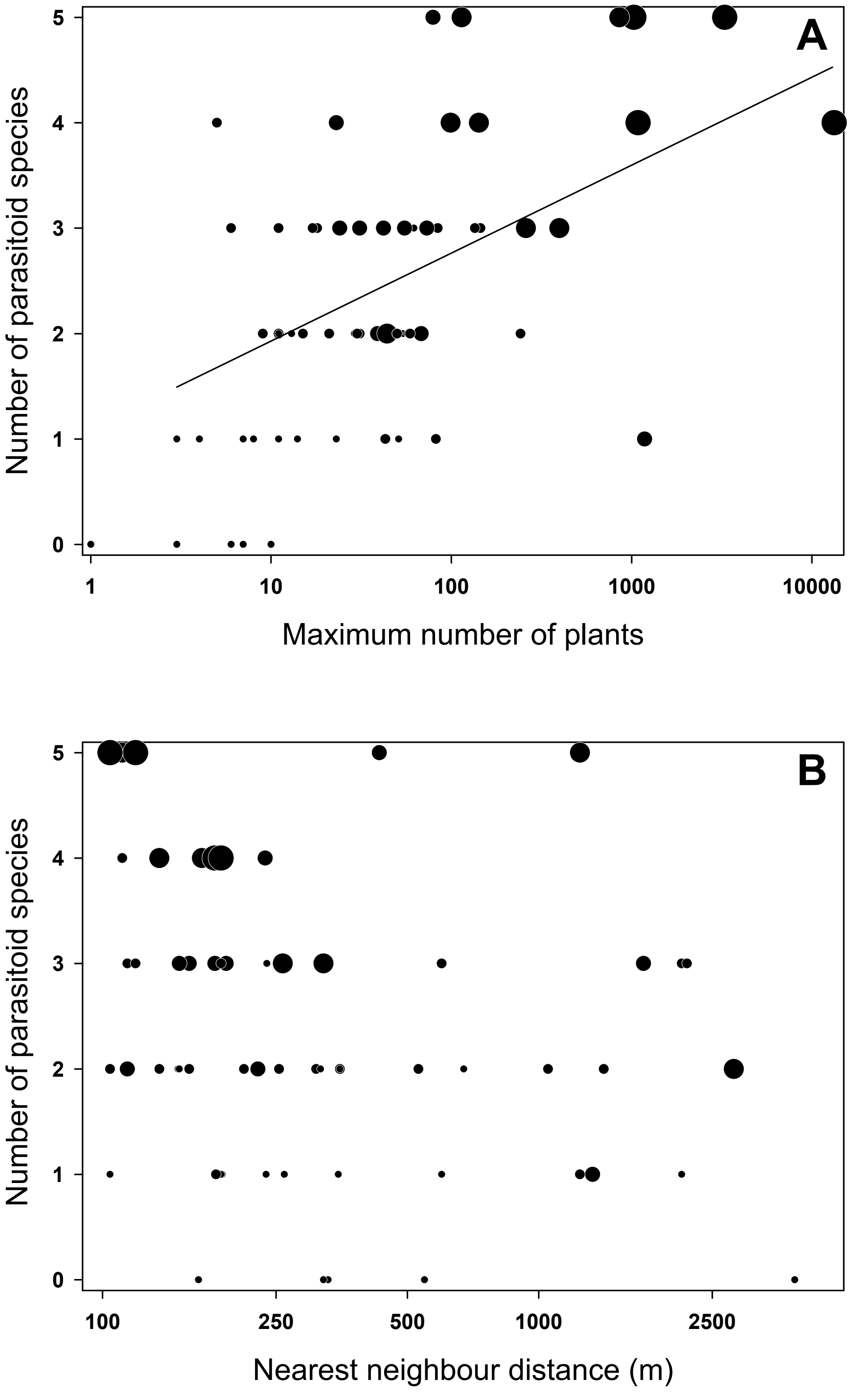

Figure 4.5 The number of parasitoid species found on *H. bicruris* during the three years in natural *S. latifolia* patches as a function of (A) patch size and (B) isolation. The size of the dots indicates the sample size of caterpillars on which the datapoint is based (see legend Fig 5.4). The line indicates the result from a linear regression with only significant variables entered.

Oviposition rate was negatively correlated with the distance of the plant from the edge (row), the strength of the edge effect differing among patches (Fig. 5.8 A, Table 5.4 A). When analysed without taking the week effects into account, two of the patches show in fact a decrease in the relative number of eggs deposited in flowers near the centre (Fig. 5.8 A). Whereas the level of herbivory decreased with distance from the edge in all patches (Fig. 5.8 B, Table 5.4 B), edge effects were inconsistent among patches for the level of parasitism; all three patches show a different relationship from positive, neutral to negative between distance to the edge and the rate of parasitism (Fig. 5.8 C, Table 5.4 C).

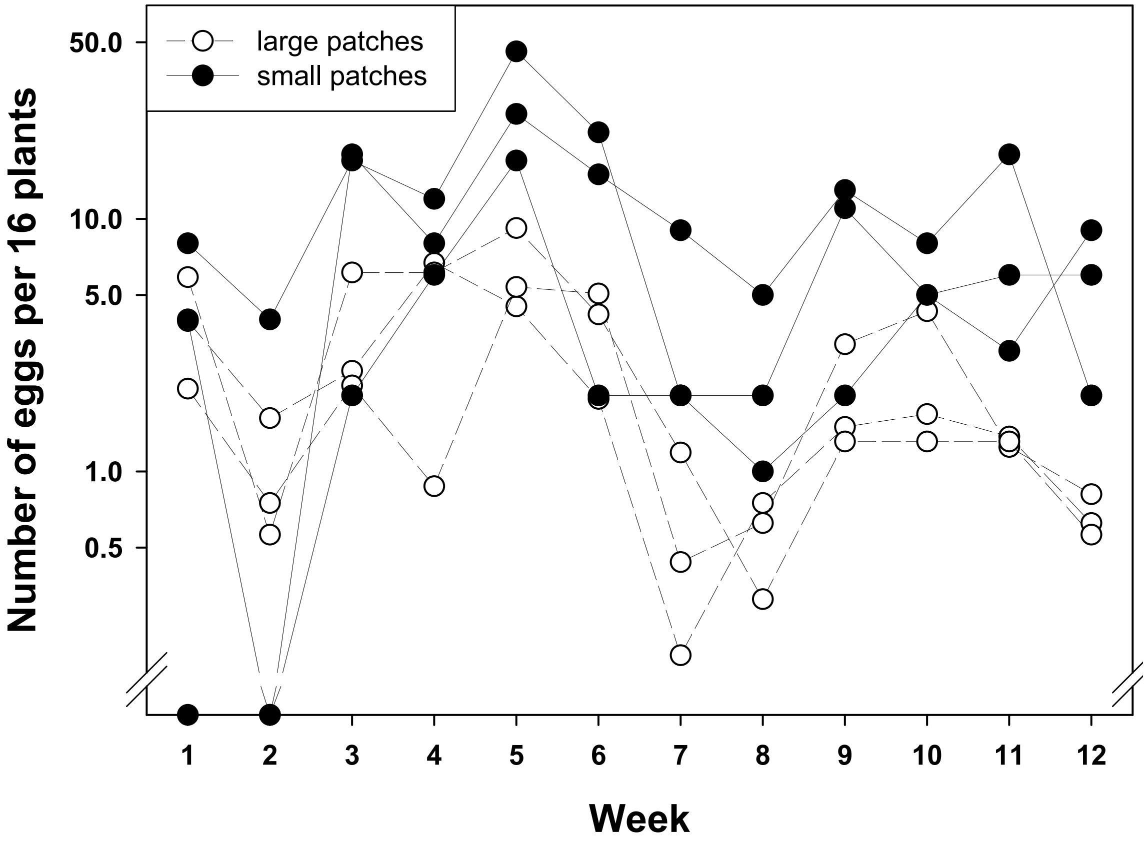

Figure 4.6 The number of eggs of *H. bicruris* on plants of *S. latifolia* in small (16 plants) and large patches (256 plants) counted weekly from May 17, 2002 until August 9, 2002 (values for large patches are averages). Note the log10 transformed y-axis.

Table 5.3 Anova table for generalised linear models explaining the proportion of flowers of *S. latifolia* in the experimental patches per week that had an egg of *H. bicruris*. Three different patch size measures were used. If the interaction is not significant, F values for the variables are derived from a model without interaction (error d.f. = 48).

A				B				C			
Variables	d.f.	F-value	*P*-value	Variables	d.f.	F-value	*P*-value	Variables	d.f.	F-value	*P*-value
Patch size (small or large)	1	4.075	0.049	Patch size (nr. of flowers per week)	1	4.944	0.031	Patch size (nr. of flowering plants)	1	4.990	0.030
Week (1-12)	11	8.319	<0.001	Week (1-12)	11	7.540	<0.001	Week (1-12)	11	8.058	<0.001
Interaction	11	*1.117*	*0.370*	Interaction	11	*1.310*	*0.248*	Interaction	11	*1.160*	*0.339*

Table 5.4 Anova table showing the analysis of edge effects (row) in the three large experimental *S. latifolia* patches on the number of *H. bicruris* eggs deposited, the level of herbivory by *H. bicruris* and the parasitism rate of *H. bicruris* caterpillars. If the interactions are not significant, F values for the variables are derived from models without interaction.

		Eggs (error d.f. = 857)		Herbivory (error d.f. = 297)		Parasitism (error d.f. = 160)	
Variables	d.f.	F-value	*P*-value	F-value	*P*-value	F-value	*P*-value
Row	1	8.324	0.004	7.846	<0.001	*0.989*	*0.321*
Week(1-12)	11	21.40	<0.001	-		-	
Patch (1-3)	2	7.140	<0.001	6.838	0.001	*0.495*	*0.611*
Row*week	11	*1.355*	*0.189*	-		-	
Row*patch	2	3.425	0.033	*0.77*	*0.464*	3.282	0.040
Week*patch	22	3.400	<0.001	-		-	
Row*week*patch	22	*1.230*	*0.213*	-		-	

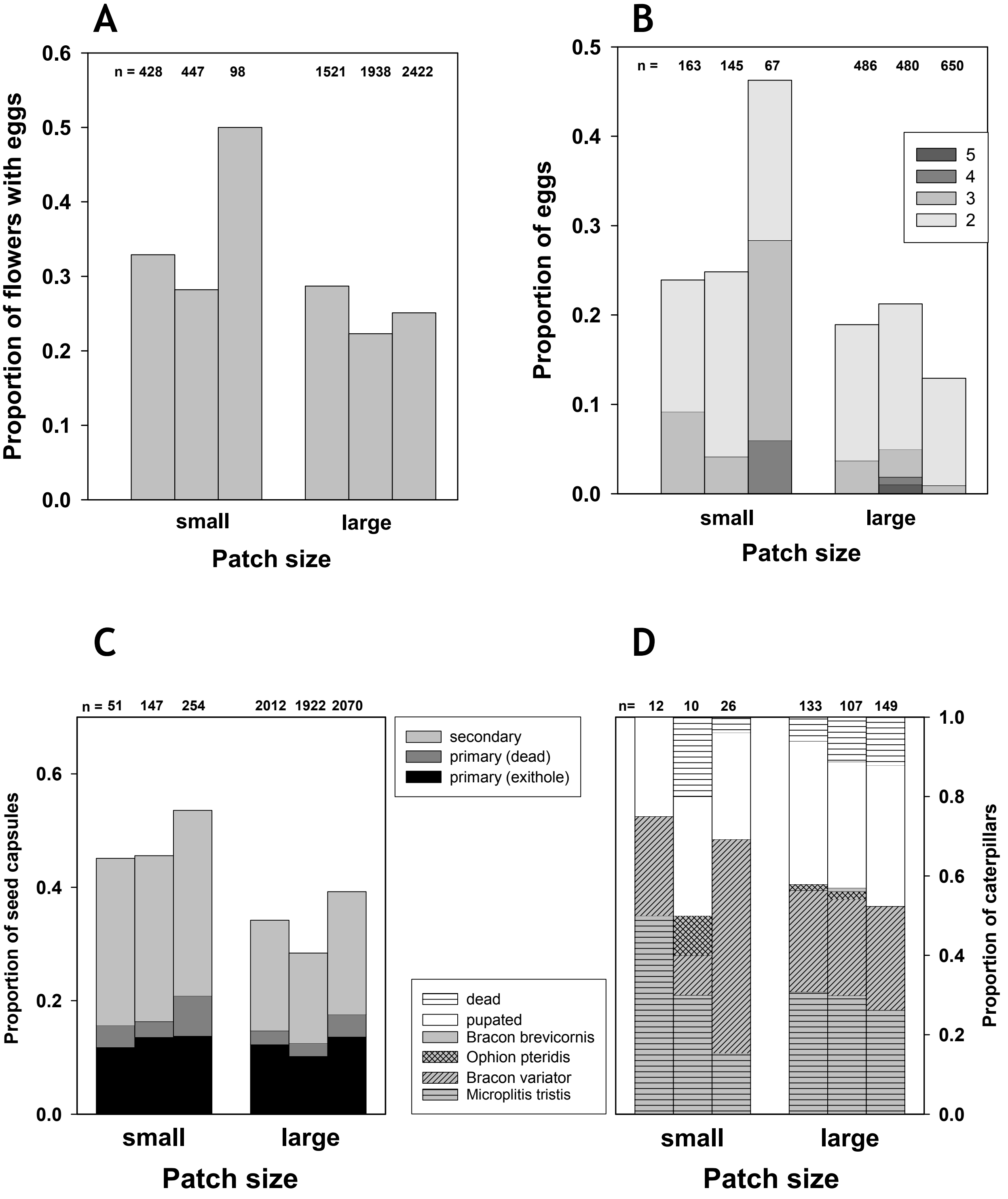

Figure 4.7 Differences between small (16 plants) and large (256 plants) *S. latifolia* experimental patches. Three replicate patches per treatment. (A) Proportion of flowers that had received an egg of *H. bicruris.* (B) Proportion of eggs in flowers with multiple (2-5) eggs. (C) The proportion of seed capsules damaged by *H. bicruris,* divided in primary (with large exit hole or with a dead caterpillar inside) and secondary herbivory. (D) The fate of all collected caterpillars from small and large *S. latifolia* patches. Caterpillars that died without any sign of parasitism are assigned to the group "dead". In all cases n refers to the total number of items investigated per patch.

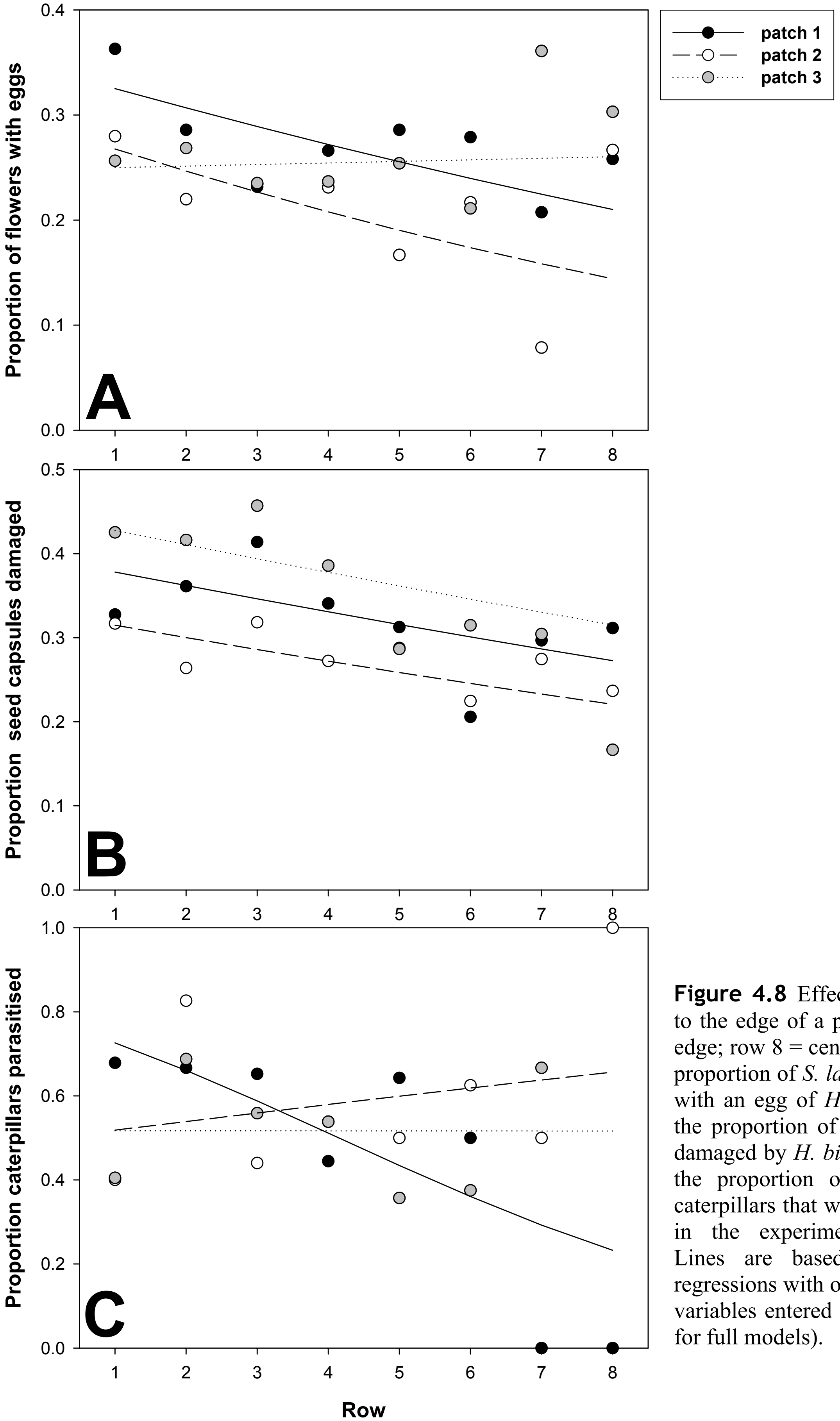

Figure 4.8 Effects of distance to the edge of a patch (row 1 = edge; row 8 = centre) on (A) the proportion of *S. latifolia* flowers with an egg of *H. bicruris*, (B) the proportion of seed capsules damaged by *H. bicruris* and (C) the proportion of *H. bicruris* caterpillars that were parasitised in the experimental patches. Lines are based on logistic regressions with only significant variables entered (see Table 5.5 for full models).

Discussion

Effect of patch size and isolation on herbivory and parasitism in natural patches

H. bicruris was present in almost every natural *S. latifolia* patch in each of the years. This result is consistent with earlier findings (Chapter 4) where *H. bicruris* was able to colonise small experimental plant patches located at least two kilometres from the neareast source population within one generation. This makes our results different from many other studies on the effects of habitat fragmentation on herbivory, where the level of herbivory decreases substantially due to the absence of less well dispersing herbivores in small or isolated plant patches (Groom 2001, Kery *et al.* 2001, Colling and Matthies 2004).

However, it is clear from our results that in fragmented populations the level of herbivory on *S. latifolia* increases. In each of the years we see an increase either in the proportion of damaged plants, number of plants, seed capsules or both with decreasing patch size and with increasing isolation. Several studies of natural systems have shown effects of habitat fragmentation on herbivory, either in a similar direction as we observe (Braschler *et al.* 2003, Lienert and Fischer 2003) or in the opposite direction, a decrease in herbivory in small or isolated patches. The latter is often attributed to corresponding differences in plant density, plant characteristics or Allee effects (Kery *et al.* 2001, Ehlers and Olesen 2003, Colling and Matthies 2004).

We can ask whether the increase in herbivory with increasing fragmentation is partly due to a decrease in the rate of parasitism, as is suggested in several studies (Roland 1993, Kruess and Tscharntke 1994). In our system it is clear that the increase in herbivory with decreasing patch size is indeed accompanied by a decrease in the number of parasitoid species and the rate of parasitism. In other studies such a decrease in parasitoid species number and rate of parasitism with decreasing patch size has been observed as well (Segarra-Carmona and Barbosa 1992, Kruess and Tscharntke 2000).

However, we argue that neither the lower number of parasitoids nor the lower rates of parasitism might make a significant contribution to the higher levels of herbivory in smaller patches. The effect of the number of parasitoid species on the total parasitism rate has rarely been studied, but is probably very species specific (Doak 2000). In our case it seems unlikely that effects on species number have a major impact on parasitism rate. Only four parasitoid species account for 95 % of the parasitisms, while all ten other species parasitising *H. bicruris* in the study area each cause only a very small number of parasitisms (Chapter 2). For only one out of these four main parasitoid species was its presence affected by isolation (Chapter 4), which may explain why we did not find any effect of isolation on the number of parasitoid species or on parasitism rate. This is in contrast with other studies which show a decrease in parasitoid species number and parasitism rate with increasing isolation (Kruess and Tscharntke 1994).

Since parasitism rates are lower in smaller patches, this could lead to increased levels of herbivory in small patches. However, the effects of parasitism on herbivory by a direct reduction of the quantity of food eaten by individual caterpillars may be small. 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 4.9 % of the caterpillars is an ectoparasitoid immediately arresting host growth and feeding, the others are koinobionts allowing the caterpillar to continue to feed and grow until just prior of eclosion of the larvae (Chapter 2). Whereas the gregarious parasitoid *M. tristis* can reduce host food consumption, at least in smaller than average clutches (Elzinga *et al.* 2003), *O. pteridis* and the larval-pupal parasitoid *E. tristis* eclose just before or after pupation possibly having little effect on host food consumption. Also, the consequences of the indirect effect of parasitism on herbivory by reducing 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 patches may well be limited by the available number of flowers rather than the number of herbivores. One female herbivore escaping parasitism can probably easily deposit an egg in all flowers present in small patches as they can produce a few hundred eggs in their life and 50 eggs per night (Brantjes 1976b, Elzinga *et al.* 2002). Also, losses in local herbivore populations due to parasitoids could be compensated by immigration of moths from nearby populations. Although the number of parasitoid species and the rate of parasitism are thus lower in smaller patches, this does not necessarily make a significant contribution to the observed higher levels of herbivory.

An interesting issue that is especially relevant for seed-predation is the possible occurrence of pollen limitation in fragmented habitats, reducing the number of produced seeds per seed capsule (Jennersten 1988, Ågren 1996) and causing the seed-predator to attack more but smaller seed-capsules. Other factors that have been proposed to explain an increase in herbivory are a reduced resistance of plants to herbivores in small populations due to inbreeding effects (Ivey *et al.* 2004). Although there is evidence that *S. latifolia* shows inbreeding effects causing increased susceptibility to a fungus, *M.*

violaceum (Ouborg *et al.* 2000), this has not been studied in wild populations, nor has the defence against the seed predator *H. bicruris* been studied. It might also be that plant resistance to herbivores is lower due to low quality of the environment, the reason why most small populations might be small in the first place.

Effects of patch size on herbivory and parasitism in experimental patches

In the natural patches many factors like the biotic and abiotic environment, density of plants, herbivores, parasitoids and genetic background of the plants might be correlated with the level of fragmentation (Debinski and Holt 2000). Our experimental patches only differed from each other in patch size. There could be no previous building up of herbivore or parasitoid populations in the patches; in both years all species had to arrive from closely neighbouring plant populations in our experimental garden or further away. Still, the rate of parasitism and the level of herbivory as observed in the experimental patches were similar to that observed in the natural *S. latifolia* patches. Only one of the common parasitoid species in the study area was not present, the larval pupal parasitoid *E. tristis*, which happens to be the species whose presence is most affected by fragmentation (Chapter 4).

Although the number of replicates per patch size was low and not all effects were statistically significant, all trends were pointing in the same direction. In 2002, the number of flowers and flowering plants in a patch had a negative effect on oviposition rate, leading to a relatively higher number of eggs deposited in small patches. Also, the level of herbivory of seed capsules in 2003 was higher in small patches, which may have been mainly due to a higher proportion of flowers on which an egg was deposited. Although the level of herbivory might have been lowered by parasitoids, especially by the ectoparasitoid *B. variator*, there is no indication that parasitism rates were higher in large than in small patches. This suggests that the difference in the level of herbivory between patches of different sizes was not primarily caused by parasitoids, but more due to a behavioural response by the moth leading to higher proportions of flowers and plants with *H. bicruris* eggs in small patches.

Whereas both moth oviposition rate and the level of herbivory decreased with distance from the edge of a patch, the parasitism level did not show a clear corresponding pattern. Similar to our study, Mcgeoch and Gaston (2000) show that the prevalence of a leaf miner increases near woodland edges but that there is no change in parasitism rate. Again, this may indicate that changes in the level of herbivory are the result of changes in the behaviour of the moth resulting in different oviposition rates. Several other studies have also documented edge effects on herbivory. Most of them point in the direction of more herbivores and more herbivory at the edges of patches (Lienert and Fischer 2003). This could partly be due to more favourable microclimate or vegetational conditions (Cappucino and Martin 1997) or due to a change in dispersal behaviour at habitat boundaries (Sparks and Parish 1995, Fagan *et al.* 1999). Contrary to our findings, several studies showed that depending on the surrounding vegetation, parasitism rate was higher near the centre of a patch (Tscharntke *et al.* 2002, Cronin 2003a).

The higher oviposition rates and level of herbivory near edges may contribute to the higher level of herbivory level in small patches. In our experimental set-up the small patches have a relative large edge:interior ratio. Also other studies have reported an increase in oviposition rate in small patches due to a higher proportion of edges (Cappucino and Root 1992). Whether these edge-effects play a role in natural patches of *S. latifolia* is unknown. The patches have very different edge:interior ratios due to their different shapes, from almost circular to linear. Furthermore, it will be difficult to distinguish edge-effects from plant density effects, as density often changes near the edge of a plant patch.

Effects of habitat fragmentation on the tri-trophic system

From our study it is clear that fragmentation affects the level of herbivory by the specialist noctuid *H. bicruris*. A decrease in patch size, a higher degree of isolation and an increase in edge-effects are all likely to increase the level of herbivory. Although especially patch size in the natural patches affects parasitism rate and the number of parasitoid species, it is in this system not likely that it causes the differences in herbivory level. We speculate that behavioural responses of the herbivore to patch size, leading to differences in oviposition rate may be more important in this system. This was also suggested by Dempster *et al.* (1995a) who did not find indications for regulatory effects of parasitoids on population size of the herbivore in a patchy environment but a strong bottom up effect of plant patch size.

Several mechanisms may cause the difference in egg-laying behaviour in small versus large plant patches. First, adult moths might be relatively more attracted by small plant populations. It might also be caused by changes in patch or host plant leaving decisions in individual moths. This would mean that in small or isolated patches the moth stays relatively longer and visits relatively more flowers before it leaves the patch or plant, similar to the observation that in isolated patches pollinators may stay longer (Schulke and Waser 2001). As indicated by the higher level of superparasitism (Brantjes 1976a), the acceptance rate of flowers by the moth of lower value might also become higher in small or isolated patches. The last two mechanisms could be caused by the relatively larger numbers of mature eggs present in a female moth in isolated or small patches. A moth can produce during one night 40 eggs (Brantjes 1976b, Elzinga *et al.* 2002), much more than the number of female flowers that is available in small populations. Also, an increase in herbivory can occur near edges when herbivores hesitate to cross patch boundaries (Cantrell and Cosner 1999). Whether this behavioural response of *H. bicruris* to patch size and/or habitat edges has an adaptive value is unknown but in small natural patches survival chances of larvae might be higher due to a lower parasitism rate. Courtney and Courtney (1982) suggested that edge-effects in oviposition rate by Pierinae butterflies are the result of searching behaviour adapted to plants that occur with an overall low density.

Whether the increased level of herbivory in small populations will affect plant population dynamics is not known. In our research period only very few patches 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 patches may be more important than the effect of a reduction in seed set due to herbivory. Nevertheless, in several small natural plant patches seed set was reduced to zero by herbivory, which obviously may have consequences for the population viability.

Our research 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 patches are located by the herbivore, damage can be increased due to fragmentation, leading to reduced plant population viability.

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Chapter 6

Age dependent clutch size in a koinobiont parasitoid

with Jeffrey A. Harvey and Arjen Biere

accepted in Ecological Entomology

Abstract

The Lack clutch size theory predicts how many eggs a female should lay to maximise her fitness gain per clutch. However, for parasitoids that lay multiple clutches it can overestimate optimal clutch size because it does not take into account the future reproductive success of the parasitoid. From egg-limitation and time-limitation models, it is theoretically expected that (i) clutch size decreases with age if host encounter rate is constant, and (ii) clutch size should increase with host deprivation and hence with age in host-deprived individuals.

Clutch sizes produced by aging females of the koinobiont gregarious parasitoid *Microplitis tristis* that were provided daily with hosts, and of females aging with different periods of host-deprivation were measured.

Contrary to expectations, during the first two weeks, clutch size did not change with the age of the female parasitoid, neither with or without increasing host-deprivation time. After the age of two weeks, clutch size decreased for parasitoids that parasitised hosts daily. The decrease was accompanied by a strong decrease in available eggs. However, a similar decrease occurred in host-deprived parasitoids, that did not experience egg-depletion, suggesting that not only egg-limitation caused the decrease in clutch size.

If the natural lifespan is relatively short, for koinobiont parasitoids like *M. tristis*, that have low natural host encounter rates and short oviposition times, costs of reproduction due to egg-limitation, time-limitation or others are relatively small. Koinobiont parasitoid species, that in natural situations experience little variation in host density and host quality, might not have strongly evolved the ability to adjust clutch size.

Introduction

Over many years, clutch size decisions by gregarious parasitoids have received considerable attention (Godfray 1994). One of the most influential hypotheses concerning optimal clutch size that has been used extensively in parasitoid research, although it originally focused on birds, was developed by Lack (1947). According to Lack's hypothesis, a mother should lay the number of eggs that maximises her gain in fitness from the whole clutch (Godfray 1994). For gregarious parasitoids this means that in hosts of a given quality (e.g. size or resource complement) the mother should lay the number of eggs that results in maximum fitness returns (referred to as "the Lack clutch size"). If the fitness of the parasitoid larvae in different-sized clutches can be determined, it is quite straightforward to calculate the Lack clutch size for a parasitoid, and several studies have attempted to do so (Waage and Ng 1984, Charnov and Skinner 1985, Waage and Godfray 1985, Le Masurier 1991).

Until now, most studies with parasitoids have observed that the clutch sizes were smaller than the predicted Lack clutch sizes (see Godfray 1994, Vet *et al.* 1994) either because the Lack clutch size was overestimated, or more fundamentally, because the Lack hypothesis of maximising fitness gain per clutch is incomplete (Hardy *et al.* 1992, Vet *et al.* 1994). Although Lack's hypothesis yields clear testable predictions, it does not take into account that optimising lifetime reproductive success is only equivalent to maximising the success of an individual clutch, if animals lay a single clutch in their lifetime or when opportunities to lay additional clutches are very rare (Hardy *et al.* 1992). Instead of focusing only on the role of the quality of the host, parameters like size, age, experience and egg load of the female parasitoid and environmental parameters like host density and availability should therefore also be considered when assessing the optimisation of lifetime reproductive success of a parasitoid (Godfray *et al.* 1991).

Two important constraints that might limit the lifetime reproductive success of a parasitoid are usually considered; egg-limitation and time-limitation (Rosenheim 1999). Some parasitoids emerge with a full complement of mature eggs (so-called 'pro-ovigenic' species (Jervis *et al.* 2001)), and may have a limited egg supply to maximise lifetime reproductive success. When hosts are abundant there is a risk that the female parasitoid depletes her egg supply before death (Driessen and Hemerik 1992). Alternatively, many, perhaps most, parasitoids are capable of maturing a number of eggs after eclosion (so called 'syn-ovigenic' species (Jervis *et al.* 2001)), that is probably far in excess of what is required to parasitise the number of suitable hosts that are normally encountered during the lifetime of a single female wasp. In this situation, it is important for the parasitoid to optimise the ratio between time spent for oviposition and time spent for locating hosts and host patches (Charnov and Skinner 1985, Rosenheim 1999).

Of the two constraints, it is assumed that time-limitation is much more important in natural situations due to the scarcity of available hosts and short lifespan of most parasitoids (Rosenheim and Rosen 1991, Mangel and Heimpel 1998), although egg-limitation can also play a role over the first few days following eclosion when only few eggs have matured or when host densities are high (Driessen and Hemerik 1992). Rosenheim (1999) and Rosenheim and Rosen (1991) argued that clutch size is affected by a combination of egg-limitation and host encounter rate, and that both constraints might play an important role simultaneously in determining optimal clutch size at a given time. Dynamic optimisation models that maximise lifetime reproductive success with egg-load and/or time as limiting factors predict that the optimal clutch size will vary over time in response to changes in egg load, the number of previous host encounters, and host density and availability (Iwasa *et al.* 1984, Mangel 1987, 1989, Mangel and Heimpel 1998).

There are two general predictions with regard to the effects of egg-limitation in parasitoids on changes in clutch size over time. (1) When the total number of host encounters increases, clutch sizes should decrease (Iwasa *et al.* 1984, Parker and Courtney 1984). This means that aging females that regularly encounter hosts are expected to decrease clutch sizes as the risk of egg depletion increases (prediction A) (Mangel and Heimpel 1998). (2) When the rate of host encounters increases, clutch sizes should decrease, because the risk of becoming egg-limited becomes larger. Female parasitoids aging without access to hosts over a protracted period should lay larger clutches than conspecifics with a higher host encounter rate (prediction B) (Iwasa *et al.* 1984).

Predictions for time-limitation are comparable but depend much more on specific assumptions, especially on the relationship between the risk of mortality and age (Iwasa *et al.* 1984, Mangel 1989). When a constant number of hosts is encountered, i.e. host encounter rate is constant, clutch size is not expected to change with age if life expectancy is independent of age. If life expectancy decreases with age, which is more likely, a decrease in clutch size with age is expected, similar to prediction A, as time becomes increasingly limited. When host encounter rate increases, or similarly when host-deprivation time decreases, the clutch size is expected to decrease, which is similar to prediction B (Parker and Courtney 1984).

As age is considered to influence clutch size decisions in parasitoids it is remarkable that so few experimental studies have investigated in detail the effects of age on clutch size in gregarious parasitoids. Different studies, all with excess numbers of hosts provided, show some support for prediction A. Bezemer and Mills (2003) and Melton and Browning (1986), found that clutch size decreased with age for two species of idiobiont ectoparasitoids. The most complete studies have been done on *Cotesia glomerata*, a koinobiont parasitoid that habitually attacks both solitary and gregariously occurring hosts. It has been shown that clutch size decreases during the first three parasitisms (Ikawa and Suzuki 1982, Vos 2001) but stabilises thereafter (Ikawa and Suzuki 1982), supporting prediction A only weakly. Unfortunately, all mentioned studies consider only a relatively short time period.

Prediction B has been only partially confirmed. Although several studies show that idiobionts or ectoparasitoids may reduce clutch size with increased host densities and host encounter rates (Rosenheim and Rosen 1991, Nakamura 1997, Zaviezo and Mills 2000), detailed studies with different host-deprivation times or taking age into account are lacking. Studies performed with *C. glomerata* showed changes in clutch size in response to host encounter rate, giving some support to prediction B (Ikawa and Okabe 1985, Tagawa 2000).

The study presented in this paper does not focus on the Lack clutch size theory, but it investigates predictions of clutch-size models that can be translated into predictions of clutch size in relation to the age of the female parasitoid. This is one of the first studies to examine the effects of age in a gregarious koinobiont parasitoid on clutch size. In addition, the clutch sizes of females provided daily with hosts are compared with those of females being deprived of hosts up to different ages. Furthermore, the occurrence of egg-limitation was checked by measuring egg-reserves in deprived and non-deprived aging females.

Based on the predictions of the time and egg-limitation models and former studies, it is expected that (I) when female parasitoids are daily provided with hosts, i.e. age increases with a simultaneous increase in the number of host encounters, the clutch size will decrease; (II) that these clutch sizes are smaller than those of parasitoids being deprived of hosts up to a similar age, and (III) that, if the time of host-deprivation before the first parasitism, i.e. age increases without host encounter, clutch size will increase.

For the experiment, *Microplitis tristis*, a gregarious koinobiont parasitoid that is a specialist on its lepidopteran host *Hadena bicruris* was used. It is discussed whether the results support the time-limitation and/or egg-limitation theory for species that lay multiple clutches and face dynamic resources.

Methods

Species description

Microplitis tristis Nees (Hymenoptera: Braconidae) is a gregarious koinobiont endoparasitoid that is a specialist on larvae of *H. bicruris* (Shenefelt 1978). During development, larvae of *M. tristis* feed primarily on host haemolymph. When larval growth is complete, mature parasitoid larvae egress from the host by chewing a hole through the host's cuticle. The parasitoid larva then spins a cocoon next to the host and pupates.

The host, *Hadena bicruris* Hufn. (Lepidoptera: Noctuidae), is a specialist herbivore of *Silene latifolia* Krause and a few other closely related caryophyllaceous plant species (Wirooks and Plassmann 1999). The female oviposits on the ovary of female flowers (Brantjes 1976b). Directly after hatching, the first instar larva (L1) chews a small hole in the ovary, enters the young fruit, and starts to feed on the ovules and developing seeds. It will stay in the seed capsule until it has consumed all the seeds, which usually occurs when the larva has reached its fourth or fifth (hereafter L4 and L5, respectively) host instar. At this stage the larva leaves the seed capsule and starts feeding on other seed 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 (Biere *et al.* 2002). A previous study (Elzinga *et al.* 2003) showed that larger instars are the most suitable stages for the parasitoid to attack and develop in, with respect to fitness correlates like size and survival of the parasitoid larva. Accordingly, a field study showed that only larger instars are parasitised in the field (Biere *et al.* 2002). The experiment was performed with large L4 hosts to supply the parasitoid with a host stage that it naturally encounters in the field and that can provide clutches of different sizes with sufficient resources. In the field clutch size on hosts collected as L4 and L5 (n=393) ranges from 1 to 63 with an average of 18.0 ± 0.47 s.e. (Chapter 2).

Experiment

Parasitoid females, emerging from clutches originating from field collected parasitised caterpillars, were mated on the day of emergence with males by adding a female to a Petri-dish with approximately 10 males. Males also originated from field collected parasitised caterpillars and were randomly assigned to the Petri-dishes. These groups of males were used for several days (kept at 10 ºC) and then replaced by other newly emerged males.

After mating was observed, individual females were separated in numbered vials (30 ml plastic cups) and kept at 20 ºC in a growth cabinet with 16/8 hrs light/dark and 70 % humidity.

Mated females were divided randomly into several groups. One group (Regularly supplied hosts, hereafter called group R, n=39) was presented with one host every day, starting at the age of 1 day (after eclosion and mating). The other groups were given hosts on three consecutive days after being deprived for 2, 3, 4, 5, 8, 10, 15, 20, 25, and 30 days after eclosion and mating (Deprived groups, hereafter called group D2, D3, ..., D30, n per group approximately 20). By using data from three consecutive days it was avoided that conclusions would be solely based on the first clutch which could be influenced by the total inexperience of the female parasitoids. A separate group was kept as controls that did not receive any hosts (group C, n=42) for which longevity was compared to the other groups. For all female parasitoids, the day at which they had died was noted and their lifespan was calculated.

To test whether egg-availability changed over age, females of group R that died were stored in alcohol, dissected, and the numbers of eggs present in the calyx region of the lateral part of the oviducts and the basal part of the ovarioles (Jervis and Copland 1996) were counted. Similarly, eggs were counted in females that did not receive any hosts (group E) and were killed on purpose at 0, 1, 5, 10, 15, 20, 25, and 30 days.

Hosts reaching instar 4 were selected and stored several days before usage at 4 ºC. At the day of parasitism, the host larva was presented with a pair of tweezers inside the vial containing the female parasitoid. The host larva was briefly dipped in a solution containing mashed seed capsules and excrements from *S. latifolia*-fed larvae, to stimulate parasitism behaviour by the parasitoid. Before and after parasitism hosts were reared individually on artificial diet as described by Elzinga *et al.* (2002). Upon egression, parasitoid larvae were counted. If a host died before egression or if not all parasitoid larvae did egress successfully, the host was dissected and the numbers of remaining larvae were counted. Clutch size was calculated as the total number of egressed plus non-egressed larvae.

Analyses

All analyses were performed using the statistical package SPSS v.10.0 (SPSS Inc., Chicago, Illinois, USA) unless stated otherwise. Stepwise regressions were performed to estimate linear and quadratic effects of age of female wasps on clutch size for host-deprived wasps and for wasps regularly supplied with hosts. ANOVAs were employed to test effects of age, and host-deprivation time on clutch size. Age and host-deprivation time were analysed as continuous variables, group as a class variable. To accommodate non-linear effects of female age, both linear and quadratic terms for this factor were included in the models when stepwise regression indicated that the quadratic term was significant.

ANOVAs with post hoc tests were used to test differences in average clutch size values and average lifespan among the different groups. Mortality curves were analysed by fitting Weibull distributions to the data (see Jervis and Copland 1996 for references) with the survival analyses module in the statistical package STATISTICA v.6.1 (StatSoft Inc., Tulsa, Oklahoma, USA). The number of eggs present in the calyx region of the lateral part of the oviducts and the basal part of the ovarioles (Jervis and Copland 1996) for the different ages in group E were analysed with ANOVA followed by post hoc tests.

Because the clutch size data at different ages for group R were obtained from the same individuals, a repeated measurement analysis would be appropriate to establish the relationship between age and clutch size. However, because different individuals had vastly different life spans, such an analysis could not be performed. Instead, individuals were grouped in seven different lifespan classes. Time trends within each lifespan class were compared, to rule out the possibility that a change in clutch size with age as found with an ANOVA could be solely attributed to different average clutch sizes for individuals with different lifespans. In addition, regression analyses were used to test whether clutch sizes of individuals at different ages were correlated with lifespan.

Results

Clutch size with regular host provision

Clutch sizes ranged from 1 to 28 except for one exceptionally high point of 41. The latter was assumed to have been caused by a self super-parasitism and this value was therefore deleted from all further analyses. Stepwise regression showed that there was a significant non-linear relationship between clutch size and age for females that were presented with one host each day (group R; Table 6.1). Clutch size decreased with age but the decline did not start until approximately after two weeks (Fig. 6.1). The decline was not due to an overall lower average clutch size of longer-lived individuals. At young ages (day 1-5; Fig. 6.2) longer-lived individuals produced equally high average clutch sizes as shorter-lived individuals. Also, for none of the age groups a significant correlation between the eventual lifespan of an individual and its clutch size was observed (linear regressions, all *P*>0.05). Average clutch size over the first 15 day period for all groups together was 15.6 ± 0.16 s.e.

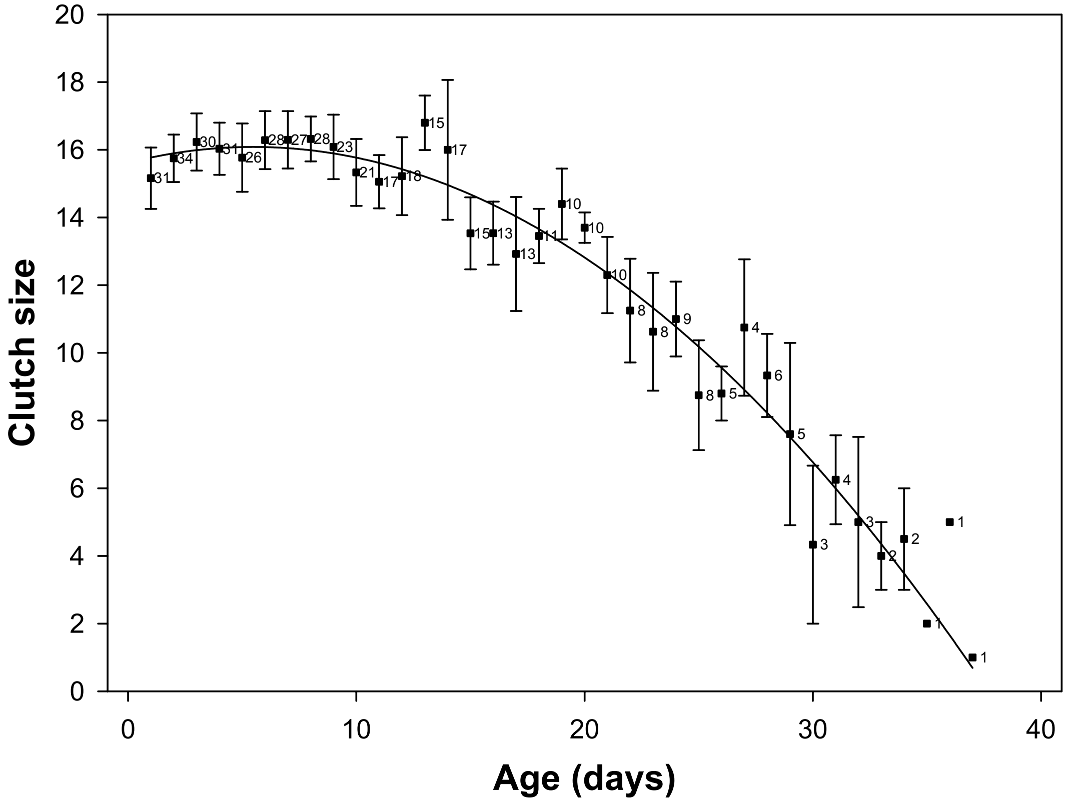

Figure 6.1 The relationship between age and clutch size for *M. tristis* females that received one host every day (group R). Dots indicate average clutch sizes (with standard error) for each age. Numbers next to dots indicate sample sizes per day. The quadratic regression line is shown. Note that due to mortality sample size decreases with age.

Table 6.1 Stepwise regression of linear and quadratic effects of age on clutch size for females receiving one host a day (group R) ($R^2 = 0.28$).

Effect	Coefficients	Std. error	T-value	*P*-value
(Constant)	15.625	0.468	33.365	<0.001
Age	0.170	0.077	2.197	0.028
Age^2	-0.016	0.002	-6.212	<0.001

Effect of host deprivation on clutch size

Comparison of the clutch sizes for parasitoids that were deprived of hosts for different periods before their first parasitism (groups R, day 1-3, and D2-D30; Fig. 6.3) showed that there was a non-linear effect of host-deprivation time on clutch size (Table 6.2) whereas there was no main or interaction effect of experience (1st, 2nd, or 3rd day after deprivation). This suggests that the first oviposition was not strongly affected by total inexperience and that only the length of the host-deprivation period had a significant effect on clutch size. Stepwise regression with only linear and quadratic effects of age showed that clutch size nonlinearly decreased with age, but again clutch size did not decrease until after two weeks (Fig. 6.3, Table 6.3). Analyses for only the first, second or third day after host-deprivation also showed significant non-linear effects of age (stepwise regressions, *P*-value for age^2 = 0.005, <0.001 and <0.001 resp.)

Table 6.2 ANOVA of the effects of experience (parasitism of the host at day 1, 2 or 3 after host-deprivation), and deprivation time on clutch size for females receiving hosts on three consecutive days after being deprived up to a certain age ($R^2 = 0.10$).

Effect	Type III sum of squares	d.f.	Mean square	F-value	*P*-value
Experience	20.215	2	10.108	0.652	0.522
Deprivation time	45.549	1	45.549	2.937	0.087
Deprivation time^2	238.540	1	238.540	15.381	<0.001
*Experience*deprivation time*	10.762	2	5.381	0.347	0.707
*Experience*deprivation time*^{2}	11.184	2	5.592	0.361	0.697
Error	9227.882	595	15.509		

Table 6.3 Stepwise regression of linear and quadratic effects of age on clutch size for females receiving hosts after being deprived up to a certain age ($R^2 = 0.14$).

Effect	Coefficients	Std. error	T-value	*P*-value
(Constant)	14.920	0.382	39.060	<0.001
Age	0.154	0.068	2.2537	0.025
Age^2	-0.0095	0.002	-4.286	<0.001

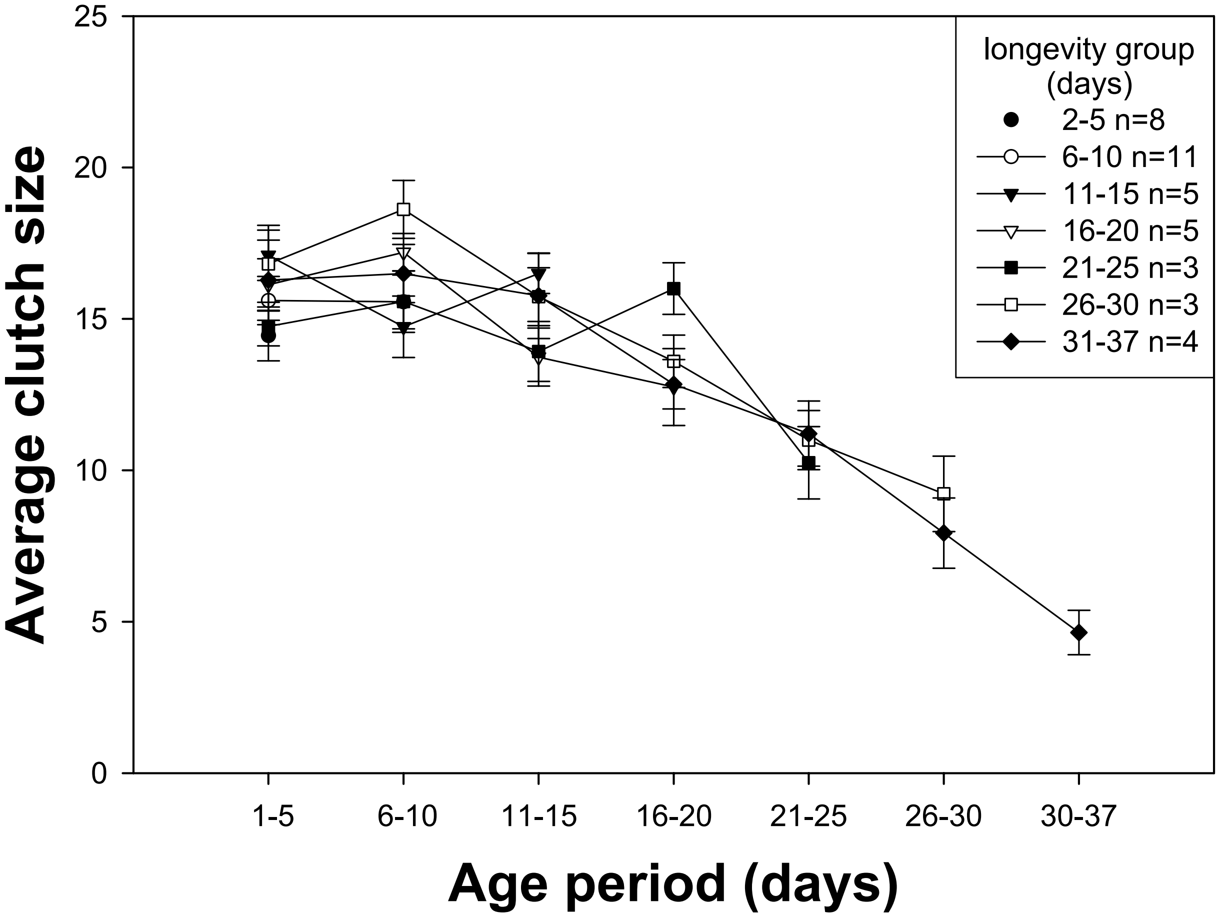

Figure 6.2 Average clutch size (± s.e.) during 5-day periods for females (group R) in groups with different longevities. This sub-division shows the relationship between age and clutch size was not due to the production of a constant small clutch size for parasitoids reaching a high age.

Comparisons between clutch sizes of host-deprived females (groups D2-D30) and females of corresponding age that daily received hosts (group R) showed that the age of females affected clutch size strongly, and that there was only a marginally significant additional effect of host-deprivation (Table 6.4). Similar analyses, where data from group R was compared with either the first, the second or the third day of the groups D2-D30, yielded similar results, namely that only age had a significant non-linear negative effect (analyses not shown). Females deprived for more than three weeks produced larger clutches than the non-deprived females of corresponding age at some ages (Fig. 6.4), but the interaction between age and host-deprivation was not statistically significant in the ANOVA (Table 6.4).

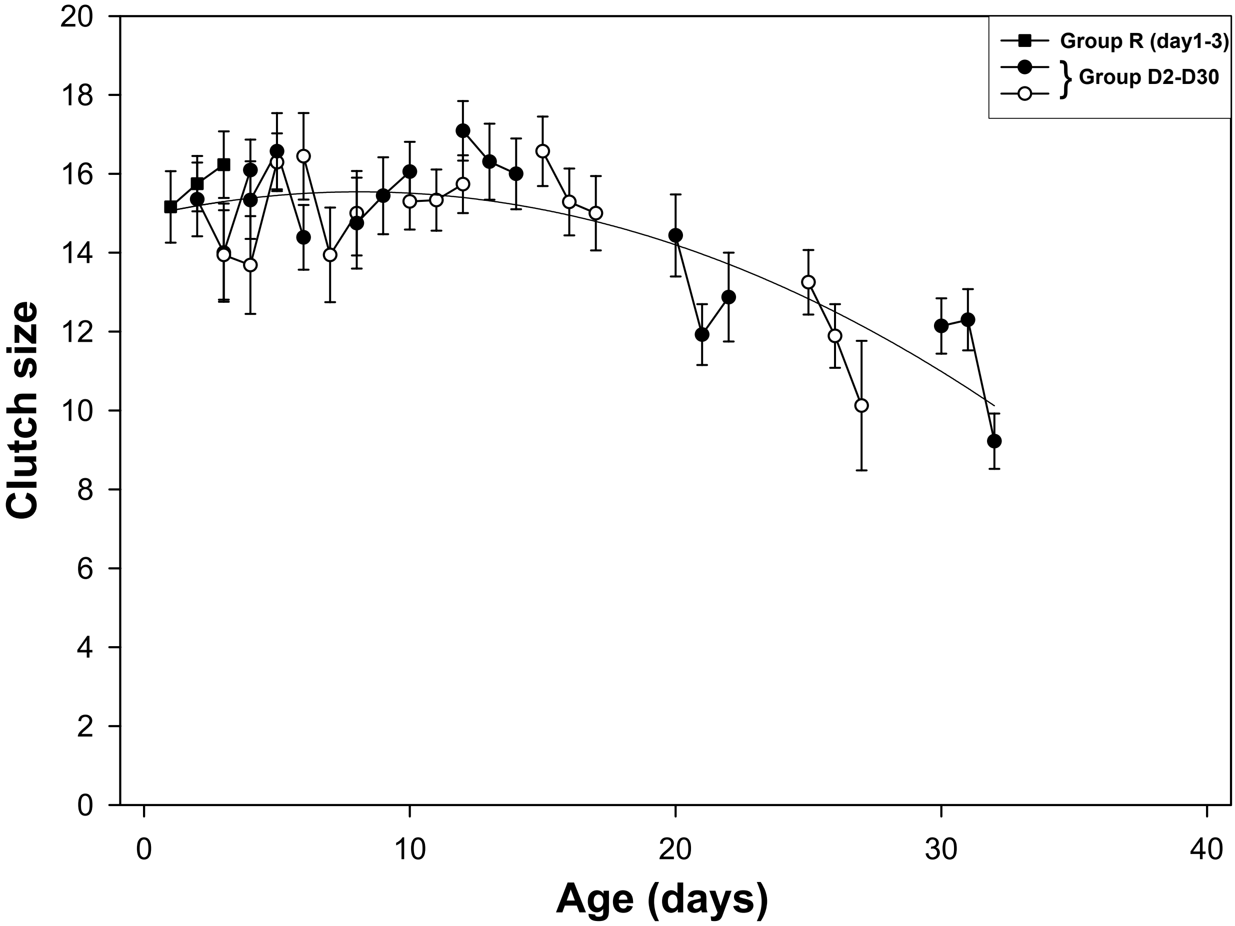

Figure 6.3 The relationship between parasitoid age and clutch size for female parasitoids that received one host every day for three consecutive days after different times of host-deprivation (D2-D30). Also, the first three parasitisms for group R are shown.

Table 6.4 ANOVA of the effects of age and host-deprivation on clutch size for all females both host-deprived (group D2-D30) and receiving a host every day (group R) ($R^2 = 0.16$).

Source	Type III sum of squares	d.f.	Mean square	F-value	*P*-value
Host-deprivation	62.477	1	62.477	3.835	0.051
Age	147.313	1	147.313	9.041	0.003
Age^2	689.231	1	689.231	42.302	0.000
*Host-deprivation*age*	6.448	1	6.4481	0.396	0.529
*Host-deprivation*age*^{2}	16.189	1	16.189	0.994	0.319
Error	14924.369	916	16.293		

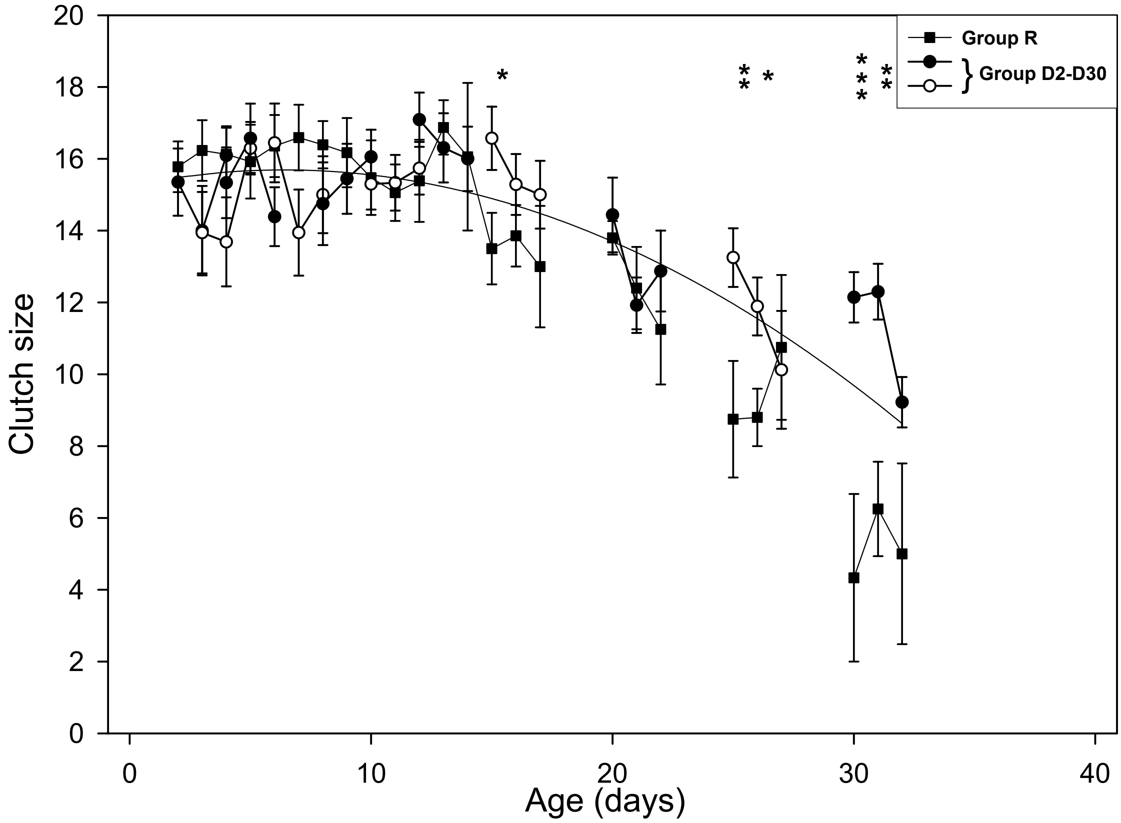

Figure 6.4 Comparison of clutch sizes between *M. tristis* females receiving one host every day (group R) and parasitoids receiving three hosts on consecutive days after being deprived for variable periods of time (group D2-D30). Data from day 1 of group R are excluded from the quadratic regression line shown. Asterisks indicate significant differences between groups for individual t-tests per age.

Longevity and egg availability

Average longevity was lowest in group R (every day a host) (Fig. 6.5). Longevity in this group differed significantly from longevity of groups D4, D10 and D20 and the controls (ANOVA with post hoc test). Figure 6.6 shows that, overall, 50% of the female parasitoids survived for up to 40 days but that 50 % of the parasitoids in group R died before the age of 20 days. The fitted Weibull distributions show that, although the best fit is observed for group R, the survivorship curves follow a type I curve sensu Jervis and Copland (1996) which indicates that life expectancy decreases with age.

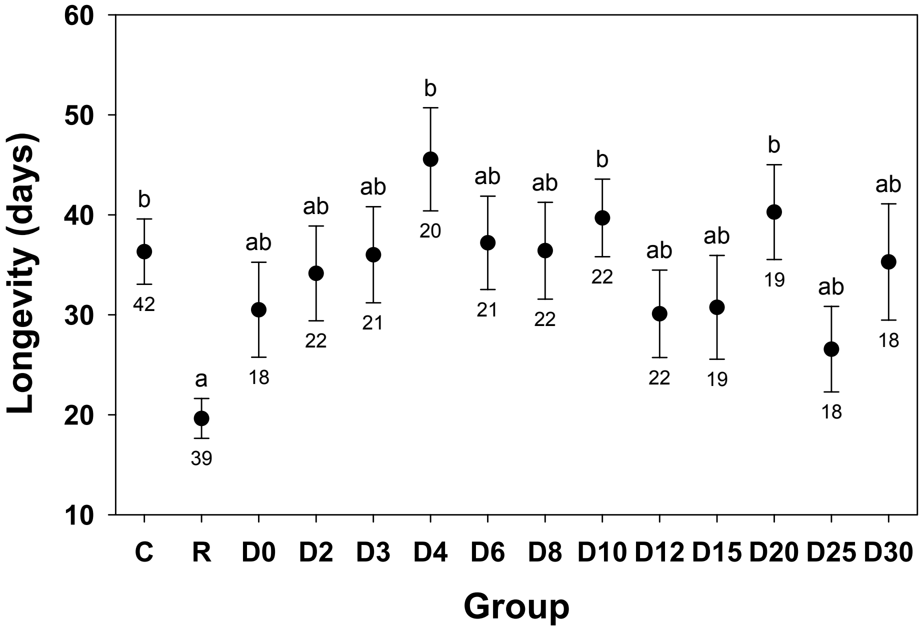

Figure 6.5 Average longevity (± s.e.) of *M. tristis* females per group (including females that died before a host was offered). Numbers indicate sample sizes per group. Different letters indicate significant differences between groups ($P<0.05$; ANOVAs with post-hoc tests).

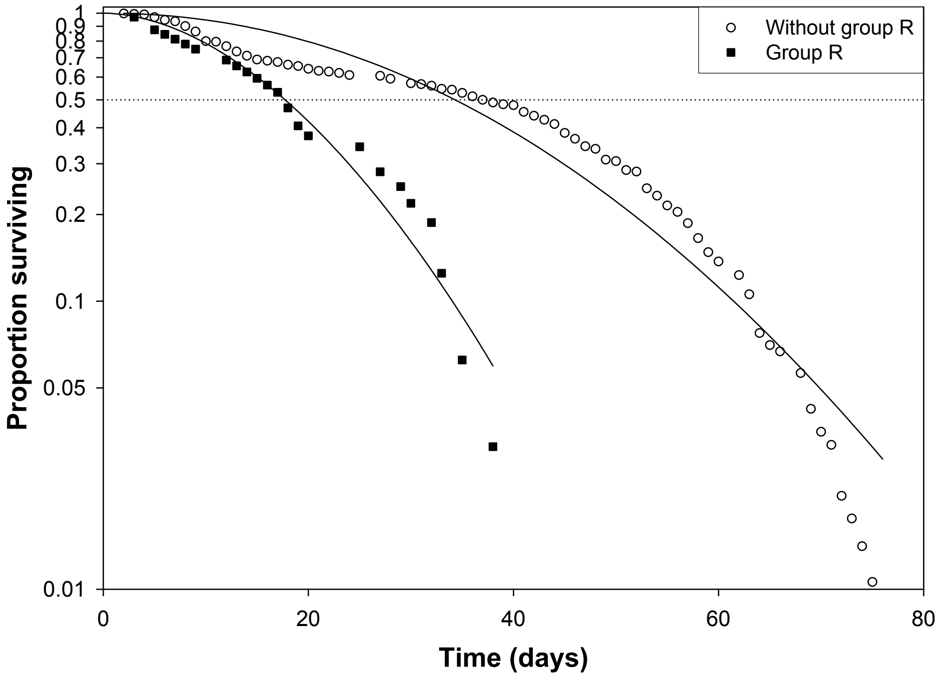

Figure 6.6 Survivorship curves of *M. tristis* females in group R and all other groups (group C & group D, including females that died before a host was offered). Fitted lines are Weibull curves. Note the log-scale on the y-axis.

The total number of eggs available (calyx region of the oviducts plus the basal part of the ovarioles) in females deprived of hosts (group E) reaches a maximum of about on average 100 eggs after a few days and does not significantly change thereafter (Fig. 6.7 A). In the calyx region of the oviducts, the maximum of on average 28 eggs is reached after 5 days (Fig. 6.7 B). Females parasitising one host per day (group R) show similar total numbers of eggs for the first two weeks, but initially seem to have on average a larger quantity present in the calyx region (Figs. 6.7 A and 6.7 B). However, for females older than two weeks, a strong decrease in the number of available eggs is noticed for the total numbers as well as for the calyx region of the oviducts (Figs. 6.7 A and 6.7 B).

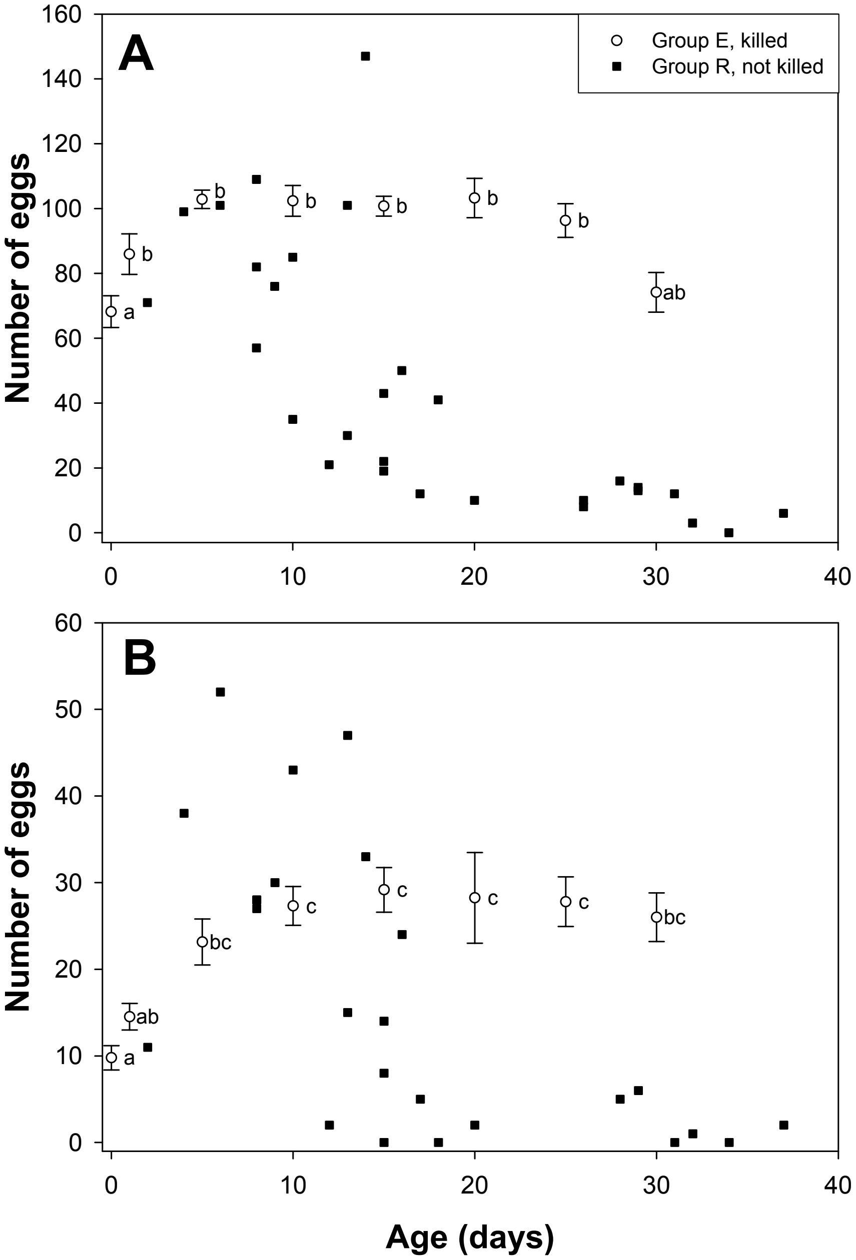

Figure 6.7 Number of eggs in dissected *M. tristis* females of group R and group E (averages and standard errors shown) in ovarioles plus oviducts (Fig. 7a), and only in the calyx region of the oviducts (Fig. 7b). Different letters indicate significant differences between ages of group E ($P < 0.05$; ANOVAs with post-hoc tests).

Discussion

The results of this study reveal that over the first two weeks of adult life there is no effect of female age on the clutch size produced by *Microplitis tristis*. This is in contrast with the prediction that with increasing numbers of hosts encountered, clutch size should continuously decrease. Furthermore, neither host-deprivation nor an increase in host deprivation time led to an increase in the clutch size produced by female parasitoids, as initially expected. Under all the different experimental circumstances the wasps produced on average the same clutch size as observed in the field. However, in more than two week old wasps from both host-deprived and non host-deprived cohorts, decreases in clutch size with age were observed. For clarity, the results for these two periods are discussed separately.

First two weeks

Theories based on egg-limitation and on time-limitation both predict that clutch size should decrease when the total number of host encounters increases, and thus clutch size should decrease with age (if life expectancy decreases with age). By contrast a decrease in host encounter rate due to an increase in host-deprivation is predicted to lead to an increase in clutch size. The underlying assumptions of both theories are discussed to speculate why the results during the first two weeks do not conform to these predictions.

The most important specific assumption of the egg-limitation hypothesis is that egg supply becomes limited at some point during the life of a parasitoid. Egg-limitation is assumed to be especially important for pro-ovigenic parasitoids or for parasitoids with a high number of host encounters. However, no known species in the Braconidae, including *M. tristis*, is strictly pro-ovigenic (Jervis *et al.* 2001). Most braconids are able to produce many more eggs during their life than the number of mature eggs they have at eclosion (Jervis *et al.* 2001). *M. tristis* is clearly syn-ovigenic, because the longest lived parasitoids in our experiment regularly supplied with hosts were able to produce more than 600 offspring, whereas the average number of eggs counted in dissected one-day old deprived parasitoids was 84 with a maximum of 156. Interestingly, our data on dissected females suggests that if females are deprived of hosts they stop producing more eggs when egg-reserves have reached a maximum threshold. Furthermore it seems that parasitism does not only stimulate egg-production but it also increases the number of eggs released into the calyx region of the oviducts, possibly increasing the number of eggs immediately available.

In an experiment with *C. glomerata*, a close relative of *M. tristis,* parasitising the solitary *Pieris rapae*, Ikawa and Suzuki (1982) observed a decrease in clutch size already after a few hosts were parasitised, indicating that egg-limitation might play a role. However, this experiment was performed with very short time intervals (1 min) between consecutive parasitisms, and might indicate that egg-production or release into the calyx region of the oviducts could not keep up with the oviposition rate rather than showing that egg-limitation was permanent. In this case the time period (1 day) between host experiences might be sufficient to replenish the complement of immediately available mature eggs, and egg-limitation could therefore only have occurred if the production of eggs within one day decreased below the average clutch size. This appears to happen after about two weeks, when egg-reserves in females regularly supplied with hosts decreased.

The most important specific assumption of the time-limitation hypothesis is that laying eggs costs time. If the time available to find hosts is limited, a parasitoid should increase her investment in time spent searching for a new host, rather than to add extra eggs to a clutch (Rosenheim 1999). When average host finding time is high because of a low host density, egg-laying time becomes relatively less important and clutch sizes should be closer to the Lack clutch size (Charnov and Skinner 1985, Skinner 1985). Although Ikawa and Suzuki (1982) found a clear relationship between clutch size and the temporal duration of oviposition in *C. glomerata*, many koinobionts that produce tiny hydropic eggs can often complete the oviposition sequence in a relatively short time, even when laying large broods. *M. tristis*, for example, can complete oviposition in less than 10 seconds. Adding extra eggs to a clutch would therefore only cost a few seconds which would not substantially decrease the chances of finding new hosts in an environment with low host densities. This is in great contrast to many ectoparasitoids, where paralysis and subsequent oviposition can take up to several hours in some species (Zaviezo and Mills 2000, Harvey *et al.* 2003). Godfray *et al.* (1991) suggested that there may be relatively few cases in which time spent on oviposition is a substantial fraction of time available for reproduction.

Both theories assume that adding an extra egg to a clutch implies extra costs either expressed in time or in eggs, resulting in decreased future reproductive success for the female parasitoid. However, other factors may be influential and would lead to similar predictions about clutch size. This may include the increased risk of mortality during parasitism due to predation or to defensive behaviour of the host (Rosenheim 1999). With several parasitoid species, including *M. tristis* (pers. obs.), hosts aggressively resist parasitism and have been observed to disable the parasitoid during oviposition (Jones and Kim 1994). It has also been suggested that the production of eggs after parasitism to replenish the stock of eggs may take energy that could otherwise have been spent to increase survival (Ellers *et al.* 2000), but whether the production of the tiny hydropic eggs in braconids will decrease survival substantially remains to be tested. Although it was observed that increased reproduction decreases longevity of *M. tristis* in this experiment, the exact cause is not known.

A more general assumption of both theories is that the female parasitoid is capable of adjusting her clutch size in response to different host encounter rates. Several studies have reported that parasitoids are able to perceive a given host density and to adjust their foraging behaviour (Roitberg and Prokopy 1983, Hardy *et al.* 1993) including clutch size (Nakamura 1997, Zaviezo and Mills 2000, Bezemer and Mills 2003). Also in *C. glomerata*, it was demonstrated that host encounter rate influences clutch size (Ikawa and Okabe 1985, Tagawa 2000) but more studies of koinobionts are necessary to broaden this observation. One explanation why host-deprivation during different periods did not lead to a higher clutch size compared to the non-deprived wasps in this experiment, may be that receiving a single host per day is already perceived by the parasitoid as an extremely low host density. The theories generally predict that all broods should approximate the Lack clutch size if the host density is very low (Parker and Courtney 1984, Charnov and Skinner 1985, Skinner 1985). However, based on the biology of *M. tristis* and *H. bicruris*, one host encounter a day or less might reflect natural host densities. *Silene latifolia*, the food plant of the host, usually occurs in separate patches consisting of only a few tens of individual plants. The host lives solitarily on the seed capsules of the plant which are produced in relatively small quantities that are distributed over time (Wright and Meagher 2003). Therefore, the number of hosts that are in a suitable stage and exposed to *M. tristis* at a certain moment in a plant patch is likely to be small, suggesting that under natural circumstances clutch size adjustment to host encounter rate could be unimportant. Further studies with higher host encounter rates could give more insight.

The most basic assumption of all the optimal clutch size models is that parasitoids are generally capable of adjusting clutch size. Clutch size adjustment is most strongly expected in parasitoids that parasitise hosts of variable size or quality and many idiobiont parasitoids are known to do so (see Salt 1961, Hardy *et al.* 1992, Godfray 1994). However, only in a few cases has clutch size adjustment been observed in koinobiont parasitoids (Sato *et al.* 1986, Schopf and Steinberger 1996). For many others, including *M. tristis*, clutch size adjustment to host size does not occur (Elzinga *et al.* 2003).

Furthermore, it seems that under natural circumstances variation in the size of hosts parasitised by *M. tristis* is relatively small (Biere *et al.* 2002, Elzinga *et al.* 2003). This suggests that *M. tristis* might not need to adjust clutch size to different host sizes under natural circumstances because the size of hosts upon parasitism does not substantially vary in the field. Also, koinobiont parasitoid larvae are capable of regulating the growth of different-sized hosts according to parasitoid load (Sato and Tanaka 1984, Harvey 2000, Elzinga *et al.* 2003), indicating that clutch size adjustment to host size is less important.

Parasitoids clearly have adapted to different hosts and their biologies. Some hosts will always occur in relatively high densities when living gregariously or semi-gregariously during a season. Others might occur only occasionally in high densities during outbreaks, but many hosts may always be spatially or temporally scattered. For parasitoids attacking host species that occur (ir)regularly in high densities, adaptation to a range of host densities might be expected. In these species we can expect to find effects of age and host-deprivation on clutch size. This may explain why in several studies with the parasitoid *C. glomerata,* which attacks both solitary and gregariously occurring hosts, effects of age and host densities on clutch size were found (Ikawa and Suzuki 1982, Ikawa and Okabe 1985, Tagawa 2000), while we do not find such responses in *M. tristis*. Parasitoids attacking hosts that occur in relatively constant low densities have presumably adapted to produce clutches that on average maximise the fitness per clutch and thus approximate the Lack clutch size (Le Masurier 1991). However, a comparison of more parasitoid species with different natural host distributions will be needed to confirm this idea.

If natural host densities and the host sizes of *H. bicruris* that are encountered by *M. tristis* indeed do not vary substantially, *M. tristis* might simply not have evolved the ability to actively adjust clutch size, which would explain why the average clutch size does not vary over the first two weeks of adult life (this paper) nor with host size upon parasitism (Elzinga *et al.* 2003). We would then expect that *M. tristis* always produces the Lack clutch size for the average host size that is encountered in the field. A study that determines the Lack clutch size in hosts with a variety of sizes upon parasitism and detailed field observations of the average host size that is attacked are necessary to test this assumption.

After two weeks

In *M. tristis* it appears that clutch size decreases after approximately two weeks of age. Rosenheim (1999) suggests that egg-limitation might become increasingly important relative to time-limitation in later life. The observations on the egg-reserves in dissected females show that *M. tristis* indeed becomes egg-limited after parasitising about 15 hosts. The egg-reserves in these wasps are so small that it seems that they inject almost every available egg into a host, using only newly matured eggs in a subsequent host. However, a decrease in clutch size (although somewhat smaller) was also observed in the host-deprived wasps that did not become egg-limited at all. This suggests that older parasitoids may not only be egg-limited but that also other factors may play a role at high ages. It can only be speculated whether the decrease would be adaptive, e.g. a response to a relative increase in the costs of ovipositioning with age, or more a physiological result of aging.

Whether these ages and a concomitant decrease in clutch size are realistic in natural situations remains uncertain. No studies have directly compared parasitoid longevity in the laboratory with lifespan under natural situations. However, observations in the field suggest that lifespan is strongly reduced by predators (Rosenheim 1998). A study with two aphid parasitoids, *Aphytis sp.*, revealed that longevity is reduced from several weeks in the laboratory to only a few days in the field due to predation (Heimpel *et al.* 1997). Other factors that might extend longevity unnaturally in the laboratory are reduced mobility in closed environments in combination with a constant supply of a carbohydrate source such as honey (Jacob and Evans 2000). This suggests that *M. tristis* individuals of more than two weeks old, might be rarely present in natural situations.

Conclusions

In summary, it appears that clutch size in the koinobiont *M. tristis* does not vary with the duration of host-deprivation nor with age during a time span of approximately two weeks. The results suggest that *M. tristis* produces on average the same clutch size under a wide variety of host densities. It can be suggested that low natural host encounter rate, short oviposition times and a limited range in host size upon parasitism, has not strongly favoured the evolution towards the ability to adjust clutch size. Most clutch size models that predict different clutch sizes under egg- or time-limitation might be less relevant for koinobiont parasitoids that experience low host encounter rates and short oviposition times under natural conditions. Only extremely old *M. tristis* parasitoids might be limited in the clutch sizes that they produce due to a reduction in egg-production or some effect of aging. When predicting effects of age on clutch size for koinobiont parasitoids, careful attention should be paid to the natural biology of parasitoids and their hosts, including natural (variations in) host encounter rate and host quality.

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Chapter 7

Summarising discussion

The main aim of this thesis was to study the effects of habitat fragmentation on a plant-herbivore-parasitoid system. More specifically, I wanted to test the hypothesis that the presence of parasitoids is more negatively affected in fragmented habitat than the presence of the herbivore and whether that results in a negative effect on the plant due to increased herbivory. The results obtained in this research have already been discussed extensively in the preceeding chapters. In this final chapter I will give a short overview of the main results and discuss what they show about the different effects that habitat fragmentation has on the *Silene latifolia*-*Hadena bicruris*-parasitoid system. Finally, I present the general conclusions for the system and I indicate what the results contribute to the knowledge on effects of habitat fragmentation for plant-herbivore-parasitoid systems.

The effect of habitat fragmentation on the presence of the herbivore and its parasitoids

One of the main effects expected from habitat fragmentation is an increased number of habitat patches where a species is absent. Due to a decrease in patch size, a decrease in connectivity and an increase in edge:interior ratio, the probability of extinction of local populations may become increased whereas the probability of recolonisation of a patch might become decreased (Hanski and Gilpin 1996, Hanski 1999). Therefore it was expected that in patches with a higher degree of fragmentation the herbivore and its parasitoids were more often absent.

The results of chapter 4 and chapter 5 show however, that the herbivore was present, at least as a larva, in basically every single patch in all three years. Even on solitary female plants, the smallest patch size, herbivory was observed in most cases. Neither size nor connectivity of the *S. latifolia* patches in the study area limited the occurrence of herbivory by *H. bicruris*. These results are different from many other studies on insect herbivores. In several studies that were performed on similar (i.e. several hundred meters up to a few kilometres distance between patches) or even over smaller scales, the occurrence of insect herbivores was negatively influenced by the level of fragmentation (Groom 2001, Kery *et al.* 2001, Colling and Matthies 2004).

From the colonisation experiment in chapter 4 it appears that the dispersal ability of the moth is very good. Almost every experimental plant patch at distances from 125 m to 2 km was colonised within a year. With such good colonisation abilities it can be expected that even if local extinction of the moth occurs in natural *S. latifolia* patches, recolonisation is also frequent, such that it is unlikely that any effect of the current level of fragmentation on the occurrence of the herbivore will be observed. From several other studies it is known that noctuid moths of similar size and shape can be strong dispersers, capable of travelling tens of kilometres in their life time (Förare and Solbreck 1997, Schneider 1999). I think that *H. bicruris* forms a single population or a few large populations in the study area and that it experiences all the *S. latifolia* patches as one large but patchy habitat, where the resources are distributed heterogeneously. In other studies on the distribution of moths in habitat patches with similar levels of fragmentation, a similar conclusion has been drawn (Förare and Solbreck 1997).

Although less well studied, parasitoids in general are believed to have much smaller dispersal abilities than their herbivore hosts (Roland 1993, Kruess and Tscharntke 1994, Zabel and Tscharntke 1998, Komonen *et al.* 2000, Kruess and Tscharntke 2000, Tscharntke and Brandl 2004). Furthermore, they may be more prone to local extinction due to smaller and more variable population sizes (Kruess and Tscharntke 1994, 2000, van Nouhuys and Tay 2001). Therefore, it was expected that the presence of parasitoids in the system would be more affected by habitat fragmentation than the presence of the herbivore. Indeed the number of parasitoid species present in patches decreased with increasing fragmentation (Chapter 5). However this effect was not due to the decreased connectivity but only to decreased patch size. As patch size is strongly positively related to the number of hosts in a patch (Chapter 4), it suggests that host population size strongly determines how many parasitoid species can be found in a patch. Most parasitoid species were rare on *H. bicruris* and/or were generalists that could use other hosts as well (Chapter 2), suggesting that their population dynamics are probably not strongly influenced by fragmentation of the *S. latifolia* patches.

Looking in more detail at the distribution patterns of the three most important parasitoid species, it appears again that the occurrence of all three parasitoids is positively related to the size of a patch (Chapter 4). Still, the specialist gregarious endoparasitoid *Microplitis tristis* is found in many patches consisting of less than 10 plants. Its presence was unrelated to most connectivity parameters, suggesting a good dispersal ability. The colonisation experiment in chapter 4 showed that colonisation of small patches by this parasitoid was frequent but independent of distance to the source, at least up to the maximum covered distance of 2 km, confirming this idea. The presence of the generalist gregarious ectoparasitoid, *Bracon variator,* was also not related to connectivity parameters. This could be expected if alternative hosts are present in the surrounding landscape. Although this parasitoid is frequently found in small patches, the smaller colonisation rates and its absence in some large populations suggest that it indeed utilises alternative hosts.

Only one parasitoid, the solitary specialist *Eurylabis tristis,* seems to be more strongly affected by fragmentation. It was more often absent from patches of small and intermediate size and its occurrence was positively related to most connectivity parameters (Chapter 4). Furthermore, it was a very poor coloniser of the experimental plant plots, showing that it probably has a lower dispersal ability.

These results suggest that some parasitoids are much more vulnerable to habitat fragmentation than others, even if they use the same host species. Several other studies have found similar results, where different parasitoid species using the same host species respond differently to the spatial pattern of the habitat (Brodmann *et al.* 1997, Roland and Taylor 1997, Doak 2000, van Nouhuys and Hanski 2002).

This study also shows that some parasitoids are capable of dispersing several kilometres, which is in contrast to several studies where negative effects on parasitoid species occurrence were already found at a scale of a few hundred meters of isolation (Kruess and Tscharntke 1994, 2000). Other work focussing on specific parasitoid species however, have shown that many parasitoids are indeed capable of dispersing several kilometres in a continuous landscape (see ref. in Godfray 1994, Jones *et al.* 1996, Goldson *et al.* 1999) but also in fragmented situations (Antolin and Strong 1987, Dempster *et al.* 1995a, Dempster *et al.* 1995b, van Nouhuys and Hanski 2002). Several characteristics of species may explain why some parasitoid species have different distribution patterns (Chapter 4). Most important ones are: larger overall population sizes, better dispersal capacities, or a lower level of specialisation. Unfortunately, for most parasitoids almost nothing is known about their dispersal abilities, while predictions of the effects that habitat fragmentation might have on their distribution patterns may strongly depend on their dispersal ability.

The effect of habitat fragmentation on the level of herbivory

Because the herbivore is omnipresent, a decrease in herbivory in fragmented patches due to its absence from small isolated patches, as observed in other studies (Groom 2001, Kery *et al.* 2001, Colling and Matthies 2004), cannot be expected in this system. Indeed herbivory was observed in practically every patch in each of the three years studied. Still, habitat fragmentation has an effect on the level of herbivory. In line with some studies (Braschler *et al.* 2003, Lienert and Fischer 2003, Perret 2003) but in contrast with others (Kery *et al.* 2001, Ehlers and Olesen 2003, Colling and Matthies 2004), the level of herbivory in *S. latifolia* patches increases with decreasing patch size and increasing connectivity (Chapter 5). Most of these studies attribute such effects to differences in plant density, plant characteristics or, for a decrease in herbivory, Allee effects (Kery *et al.* 2001, Braschler *et al.* 2003, Ehlers and Olesen 2003, Lienert and Fischer 2003, Colling and Matthies 2004). In this thesis several other possible causes for an increase of herbivory with fragmentation were investigated and discussed.

Effect of parasitism on changes in herbivory with fragmentation

With a decrease in patch size the number of parasitoid species and the level of parasitism decreased (Chapter 5), suggesting that the absence or a decreased number of parasitoids could be responsible for the observed increase in herbivory in smaller patches. In several studies it has been suggested that the negative effects of habitat fragmentation on the occurrence of parasitoids could lead to an increase of herbivory in fragmented plant patches (Roland 1993, Kruess and Tscharntke 1994, 2000).

Parasitoids can have a direct effect on the level of herbivory by reducing the amount of food consumed by a host. Idiobiont parasitoids kill or paralyse their host at parasitism, so that the host immediately stops feeding and growing. Also many koinobiont parasitoids can decrease host food consumption after parasitism (Huebner and Chiang 1982, Rohlfs and Mack 1983, Schopf and Steinberger 1996). In contrast, parasitoids that attack late developmental stages of the host do not always decrease food consumption and some gregarious koinobionts can even increase food consumption (Rahman 1970, Slansky 1978, Beach and Todd 1986, van der Meijden and Klinkhamer 2000).

In chapter 2 we see that at least 14 species of parasitoids attack almost 50 % of the *H. bicruris* caterpillars in the field. However, 95 % of the parasitisms are caused by only four species. Of these species only one, *B. variator*, is an idiobiont ectoparasitoid that paralyses its host directly upon parasitism. The other three parasitoids are koinobionts that allow the host to continue to feed and grow for various periods after parasitism. Two of them even allow the host to pupate or they emerge just prior to pupation, *Ophion pteridis* and *E. tristis,* possibly having little effect on the level of food consumption. Furthermore, all four parasitoids attack mostly large caterpillars when most of their feeding damage has already been done. In chapter 3 it is shown that the gregarious koinobiont parasitoid, *M. tristis*, can decrease food consumption markedly also in caterpillars attacked in a late stage. Whether food consumption by caterpillars in natural situations is similarly decreased by parasitism remains unclear as natural clutch sizes of *M. tristis* are on average much larger (Chapter 2) than observed in the experiment of chapter 3. It can be expected that rates of consumption will be stimulated by an increase in the number parasitoid larvae (Chapter 3) and thus a decrease in herbivory due to natural parasitism may be relatively small. In total, I conclude that the direct effect of parasitism by the whole parasitoid complex on the level of herbivory is probably quite small in the *S. latifolia*-*H. bicruris*-parasitoid system.

The indirect effects of parasitism on herbivory by reducing the population sizes of the moth are less well clear because no detailed information on population dynamics of the host or its parasitoids is available. Of course, the number of adult moths developing in a patch will be decreased when parasitism occurs. However, whether this also results in a decreased number of herbivore eggs on the plants in that patch the next generation is questionable. One female moth may produce up to 400 eggs (Brantjes 1976b, Elzinga *et al.* 2002, Bopp 2003) which is much more than the number of flowers that are available in small populations. Also, if dispersal of the moths is indeed as high as suggested in chapter 4, a large number of female moths might actually come from other plant patches. Due to high levels of migration of the moth the number of adult moths present in a patch might therefore be at least partly independent from the number of moths developing in that patch. Even if the parasitoids would be able to parasitise all caterpillars in a small patch, migrant moths could easily recolonise it and subsequently oviposit. This implies that parasitism could have little effect on the local numbers of caterpillars of the next generation.

Still, if habitat fragmentation influences the behaviour of parasitoids, interactions could change. In studies on an egg parasitoid, the number of hosts attacked per female parasitoid after arrival in a habitat patch has been shown to increase with isolation distance, and was higher near edges (Cronin 2003a, b). However, in chapter 5 of this thesis no edge or patch size effects on parasitism rate were observed in the experimental plant patches. Also, no strong effect of isolation on parasitism rates was observed in the natural populations. This suggests that parasitoids might not be strongly affected in their oviposition behaviour by fragmentation in the study system. The higher parasitism rate observed in larger natural plant patches (Chapter 5) is not necessarily caused by a change in oviposition behaviour. It is likely that the parasitoid populations in these large patches are larger and this may increase the chances that a female parasitoid is present at the same time larvae are susceptible to parasitism. *H. bicruris* caterpillars are only vulnerable to most parasitoid species when they have reached instar 4 or 5 and have left the primary seed capsule (Chapter 2, Chapter 3).

A second type of behavioural change that can occur in gregarious parasitoids is that clutch size decisions are affected by fragmentation. Although no information is available on rates of parasitoid dispersal, it can be expected that when travelling time is increased due to larger distances between patches, female parasitoid age is higher when it attacks a host. Furthermore, it may take longer for a parasitoid to encounter its first hosts in small patches where the number of suitable caterpillars may be small. In Chapter 6 I tested whether aging or host encounter rate might influence the clutch size that the most abundant parasitoid, *M. tristis,* produces. Although several theoretical models predict an effect of age or host deprivation time on clutch size decisions made by the female parasitoid (Chapter 6), no differences in average clutch sizes produced by parasitoids of differing age were observed, except for extremely old ages.

Effect of herbivore behaviour on changes in herbivory with fragmentation

If parasitoids do not cause a significant decrease in herbivory, then what causes the increased levels of herbivory with increasing levels of fragmentation as seen in chapter 5? One of the reasons could be that the herbivore has a higher oviposition rate in fragmented patches. Indeed, in several small scale studies on butterflies, positive effects of decreasing patch size or increasing edge:interior ratios on oviposition rate have been shown (Courtney and Courtney 1982, Cappucino and Root 1992). Förare and Solbreck (1997) found that egg densities and larval densities of a moth were negatively related to patch size in a study on a landscape scale.

In chapter 5 I tested whether habitat fragmentation could change the oviposition rate by *H. bicruris*. In an experiment with small and large patches it was observed that in smaller plant patches relatively more eggs are deposited per plant and per flower. Furthermore, plants near edges of patches received more eggs. Both of these effects could result in an increase in herbivory in small patches. This suggests that fragmentation may change the oviposition rate of the herbivore, which could explain why herbivory is higher in fragmented plant patches.

Other possible causes

Several other factors could potentially explain differences in herbivory due to fragmentation. First, if pollination is decreased in fragmented plant patches than seed capsules could contain less seeds and be smaller (Jennersten 1988, Ågren 1996). This would force the herbivore to eat more seed capsules. Secondly, inbreeding in small population may decrease resistance of the plants to herbivory (Ivey *et al.* 2004). Furthermore, potentially, adverse environmental conditions that have led to small patches could be a reason for plants in fragmented patches to be in bad condition, thus having less resistance to the herbivores.

These hypotheses were not investigated in this thesis, but can at least be ruled out in the experiment of chapter 5 where the oviposition rate of the moths in differently sized plant patches was measured. Therefore, I think that changes in oviposition rate of the herbivore are a likely cause of the increased level of herbivory in small and isolated habitat fragments.

Effect of fragmentation on plant population viability

Although herbivory increases due to fragmentation in this system, the question remains whether this increased herbivory is negative for the plants. Some authors suggest that it can be expected that frugivory or seed predation has a stronger effect on plant population viability than other kinds of herbivory (Lienert and Fischer 2003, Colling and Matthies 2004), but so far no studies where an increase in frugivory and herbivory was found due to habitat fragmentation investigated whether that had a negative effect on plant population viability. *S. latifolia* is perennial and it is unknown how important recruitment is in plant population dynamics. *S. latifolia* is a plant of open and disturbed habitats and it needs open vegetation with a high light intensity to persist, but also to establish (Baker 1947, Goulson and Jerrim 1997). Whether recruitment is limited due to the availability of safe sites for seeds and seedlings or seed production is unknown. One seed capsule escaping from frugivory will produce several hundred seeds (Jürgens *et al.* 2002b). The effects of seed predation on winter survival or reproduction next year of the already established plant are unknown, but seed predation can cause the redistribution of resources in above ground plant structures (Wright and Meagher 2003).

In preliminary analyses, changes in population size over the three years did not indicate any relationship to fragmentation (Elzinga, unpub. data). During our study only very few extremely small populations got extinct, and these occasions were clearly due to human disturbance. Therefore, it seems likely that changes in the vegetation due to succession or disturbance are more important for changes in *S. latifolia* population size than the level of herbivory, but to be able to study changes in plant population viability of this perennial plant many years would be necessary (Brigham and Schwartz 2003).

Vulnerability of the system to habitat fragmentation

Chapter 4 shows that the effect of habitat fragmentation on the presence of the species in the *S. latifolia*-*H. bicuris*-parasitoids system is not very strong. Only one parasitoid was negatively affected by decreasing connectivity (Chapter 4). This is in contrast with several other studies where habitat fragmentation did influence the presence of several parasitoid species strongly even at isolation distances of a few hundred meters (Kruess and Tscharntke 1994, 2000).

The good dispersal abilities of the moth and the parasitoid *M. tristis* suggest that these species can cope very well with the current level of fragmentation. There are indications that *S. latifolia* is increasing over the last decades in abundance in the Netherlands (Mennema *et al.* 1989, Werkgroep Florakartering Drenthe 1999) and that the habitat studied in this thesis is currently less fragmented than before. Due to increasing soil disturbance by road construction, clay digging, dike-renewal and restructuring of river-valleys, more areas are becoming suitable for *S. latifolia*. Also, the redistribution of river valley soil by man might introduce more patches of *S. latifolia* into the area. Seeds of the plant are also included in seed-mixtures that several local governments now use to create “natural” flowery road sides (pers. obs.). There is some, albeit scarce, evidence that within species adaptation can occur to different levels of fragmentation, mainly for body structures important in dispersal capacities (Van Dyck and Matthysen 1999). Maybe the species in the study system are adapted to a higher level of habitat fragmentation and this could possibly explain why so little effect of current fragmentation is found on the presence of most of the species.

If, on the other hand, habitat fragmentation is increasing: what would happen with the system? Although the consequences cannot completely be predicted, some hypotheses can be derived from the results obtained in this study. First, it can be expected that due to decreasing connectivity, the parasitoid *Eurylabus tristis* will be more prone to extinction. But as long as enough large plant patches will exist, the parasitoid will probably persist. Because several other specialist and generalist parasitoids are available and the fact that parasitoids may have little effect on local herbivore population dynamics and the level of herbivory, consequences of parasitoid extinction for the underlying trophic levels will probably be small. Because the most common parasitoid *Microplitis tristis* is a good disperser and may persist in very small patches, fragmentation should be considerable, before effects will be seen on this species. Other parasitoid species are generalists and their presence will not be affected, unless also the vegetation in the surrounding landscape changes.

Because *H. bicruris* seems to be a very good disperser, it is not expected that the frequency of its presence will strongly decrease with increasing fragmentation. Rather, increased fragmentation may further increase the average level of seed predation by the herbivore, independent of the loss of parasitoid species, due to increasing oviposition rates per plant. Whether this will affect plant population viability remains, however, unclear.

General conclusions

The Silene latifolia-Hadena bicruris-parasitoid system

The hypothesis that the presence of parasitoids is more negatively affected by habitat fragmentation than the presence of the herbivore and that this results in a negative effect on the plant due to increased herbivory was only partially confirmed. Although it is clear that parasitoids were more frequently absent from patches than the herbivore, which was present in all patches, the presence of most parasitoids was only affected by patch size and not by the connectivity of a plant patch. Of only one specialist parasitoid was its presence negatively affected by decreasing connectivity. The other parasitoids are either good dispersers, capable of reaching most patches in the study area, or may respond to alternative hosts.

Together with a decrease in the number of parasitoid species, the parasitism rate decreases with decreasing natural patch size. This could mean that the second part of the hypothesis, absence of parasitoids leads to increased herbivory, could hold for one aspect of habitat fragmentation, i.e. a decrease in patch size. However, although the most numerous parasitoid can have a negative effect on host food consumption in small clutches, the direct effect of parasitism by the whole parasitoid complex on herbivory is probably very small. Also, the indirect effect of parasitism through a decrease in local herbivore population size could be small. Furthermore, no indications were found that parasitoids were affected in their behaviour. The gregarious parasitoid *M. tristis* is probably not affected by fragmentation in its clutch size decisions.

Habitat fragmentation does lead to an increase in herbivory, independent of parasitoids. Most likely, this higher level of herbivory is caused by a change in behaviour of the moths in response to patch size, edge effects and possibly isolation, that leads to a higher relative oviposition rate in fragmented plant patches.

Plant-herbivore-parasitoid systems

From this research several insights have been gained that are useful when predicting the effects that habitat fragmentation may have on plant-herbivore-parasitoid systems. First, it is clear that a decrease in connectivity does not always affect parasitoid species that use the same host species to the same extent. Some parasitoid species, even if they are small, can be capable of travelling large distances of several kilometres, whereas others have limited dispersal abilities. When predicting effects of a decrease in connectivity it seems therefore very important to acquire more information on dispersal abilities and the factors that determine them, such as dispersal capacity, population sizes, or level of specialisation.

The most important aspect of habitat fragmentation in this study that influences herbivore-parasitoid-interactions is patch size. A decrease in patch size leads to a smaller number of parasitoid species and a lower parasitism rate. But even if herbivory increases with fragmentation, the two are not necessarily related. First, not all parasitoids may affect herbivory directly by decreasing food consumption of hosts, or indirectly by reducing the local herbivore population size. Secondly, if dispersal of the herbivore is high, local parasitism rate may not affect herbivore density in a plant patch.

An aspect that has received less attention in the literature is the effect that habitat fragmentation may have on behavioural changes that affect oviposition rates of species. Although I did not find evidence that oviposition decisions of parasitoids were affected, clearly oviposition rate by the herbivore did increase with patch size, proximity to the patch edge and possibly with patch isolation, leading to more herbivory in fragmented plant patches. A decrease in parasitism rate is thus not the only reason why herbivory can be increased in fragmented plant patches.

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Summary

Due to human activities the level of habitat fragmentation increases in many natural systems. Besides a reduction in habitat area, this leads to a decrease in size of the remnant patches, a decreased level of connectivity, and an increase of the edge:interior ratio. Habitat fragmentation can have several negative effects on the local populations of species in habitat fragments, which may destabilise the total metapopulation of a species.

Due to a decrease in patch size local populations become smaller which may increase the vulnerability to environmental, demographic and genetic stochasticity. A decrease in connectivity due to increasing isolation and decreasing size of surrounding habitat patches may reduce the number of immigrants. Not only does this reduce the chances of recolonisation of habitat patches but it also prevents local populations from being reinforced in number or genetic variance. The increase of the edge:interior ratio can lead to stronger environmental influences from outside the patch and animal species may change dispersal or oviposition behaviour in edges.

All these effects can make local populations more prone to extinction. However, not only these direct effects of fragmentation have to be considered. More importantly, whole communities can be disrupted since many species influence each other's population dynamics. Not only is the presence in patches of higher trophic level species dependent on the presence of lower trophic levels, they can also affect the population dynamics of these lower trophic levels. Examples are pathogens affecting their hosts or herbivores affecting their host plant.

This thesis focuses on the effects of habitat fragmentation on a tritrophic system consisting of a plant, a herbivore, and its parasitoids. Parasitoids can regulate the population dynamics and reduce the amount of food consumption of their herbivore hosts, potentially reducing the amount of damage to the plants. In several studies however, it has been found that due to habitat fragmentation parasitoids are unable to persist in remnant patches, which may consequently negatively affect the plant population if the herbivores that are less vulnerable to fragmentation are still present. In this thesis the effects of fragmentation are studied in the model system *Silene latifolia*, its specialist seed-predator *Hadena bicruris* and the different species of parasitoids that attack this herbivore. The system occurs in local patches with different levels of fragmentation in a semi-natural landscape.

In **chapter 2** I describe the complete parasitoid complex attacking the herbivore including several life history characters, such as clutch size, sex ratio and diapause. During three years caterpillars were collected from all *S. latifolia* patches and reared to detect parasitisms. At least 14 species of parasitoids were found, of which the specialist gregarious endoparasitoid *Microplitis tristis*, the specialist solitary larval-pupal endoparasitoid *Eurylabus tristis*, and the generalist gregarious ectoparasitoid *Bracon variator* were the most important. All of the parasitoids attack and develop in later stages of the host when most of the plant damage has already been caused. Many are koinobiont parasitoids and do not cause a large reduction in food consumption since they allow the host to grow and feed after parasitism. Although several hyperparasitoid species were found, they probably do not influence the parasitoid population to a large extent. The sex ratio in *M. tristis* and *B. variator* is female biased with the latter almost completely producing single sex clutches.

In **chapter 3** I investigate the specific relationship between the herbivore *H. bicruris* and the most abundant parasitoid *M. tristis*. In the laboratory, host larvae of different sizes, from early instar to almost fully grown caterpillars, were offered to female parasitoids. I measured the effect of parasitism on food consumption of the host and fitness estimates of the parasitoid such as clutch size, clutch weight, survival and development time. The results showed that the parasitoid adjusts development time and host growth to host size. In small hosts host growth was in general strongly reduced, increasing the scramble competition between parasitoid larvae and although development time of the larvae was increased and host growth increased with clutch size, parasitoid survival and size were reduced. This is in contrast with the development in larger host larvae where scramble competition was less pronounced. The optimal performance of the parasitoid in larger hosts is in accordance with field observations that show that the parasitoid attacks only large caterpillars. In the experiment, food consumption in all instars was reduced strongly by parasitism.

In **chapter 4** the effect of fragmentation on the occurrence of the herbivore and the three most abundant parasitoid species was investigated. I expected that the parasitoids would be more frequently absent from small and less connected plant patches. During three years the occurrence of the herbivore was recorded in plant patches with different degrees of fragmentation, and caterpillars were collected to observe the presences of the parasitoid species. In addition, I tested the hypothesis that parasitoids can disperse less well than the herbivore by recording the colonisation in experimental patches established at distances from 125 to 2000 meters from source populations. The results show that the herbivore is able to colonise extremely well and independent of distance at scales up to 2 kilometres, explaining why it can be found every year in all plant populations. Also, the specialist parasitoid *M. tristis* seems to be a good disperser, as its colonisation is unaffected by distance and its occurrence in the plant patches is not related to any isolation parameter. This is in contrast with the distribution pattern of the specialist parasitoid *E. tristis*, which shows that isolation negatively affects its presence. Furthermore, the probability that *E. tristis* is present in a patch strongly decreases with patch size, a possible explanation why it colonised almost none of the small experimental patches. The generalist *B. variator* showed intermediate colonisation rates and its distribution pattern was not related to isolation and only weakly affected by patch size. In this chapter I discuss different characteristics of parasitoid species, such as population size, level of specialisation, dispersal ability and colonisation ability, that may explain the different distribution patterns and responses to habitat fragmentation.

In **chapter 5** the effects of different levels of fragmentation on herbivory and parasitism are shown. In plant populations with different levels of fragmentation (i.e. size and isolation distance) the level of frugivory by the herbivore *H. bicruris* and the parasitism rate were measured. Analyses show that the level of herbivory is increased when plant population size decreases and isolation increases. A reduction in parasitism rate and parasitoid species diversity is shown with decreasing patch size but not with increasing isolation. Because the herbivore is a strong disperser and the parasitoid complex may have little direct effects on food consumption and on local host population dynamics, I think that parasitism rate has not a strong influence on the local level of herbivory. An explanation for the increased level of herbivory could be a change in oviposition behaviour in small and isolated patches. I tested this possibility by measuring oviposition, level of herbivory and parasitism rate in experimental plant patches of large and small size. Oviposition rate was indeed increased in small patches and also near patch edges. Similarly, herbivory was increased, but no effects of size or proximity to the edge on parasitism rates were discovered. The results in this chapter show that due to habitat fragmentation, plant damage can increase due to a change in oviposition behaviour of a well dispersing herbivore.

In **chapter 6** I investigate the effects of increasing age of the female koinobiont parasitoid *M. tristis* on the clutch size it produces. Based on optimal clutch size theory it was expected that with increasing age clutch size would decrease, except when females were deprived of hosts. I expected that clutch sizes in that case would be higher and also increase with increasing deprivation time. In an experiment one group of female parasitoids were offered one host per day until they died. Other females received a host on three consecutive days after being deprived for up to 30 days. The clutch sizes that were produced during the first two weeks did not change with age nor did deprivation period have any effect. In females older than two weeks, clutch sizes decreased when females had been supplied daily with hosts but also, although less strongly, when they had been deprived of hosts. Dissections showed that egg-availability strongly decreased after two weeks when hosts were provided daily, whereas it remained constant if host deprivation period increased. I argue that models that predict clutch sizes over age with different host encounter rates and that consider reproduction to be costly in terms of time- or egg-limitation, may not be relevant for parasitoids like *M. tristis* that have low natural host encounter rates and short oviposition times. It is argued that such koinobiont parasitoids that encounter relative little variation in host density and host size, might not have strongly evolved the ability to adjust clutch size.

In **chapter 7** the results of the studies presented in this thesis are summarised and discussed. The most important effect that habitat fragmentation has on the system is the increase in herbivory in isolated and small patches, probably due to a change in herbivore oviposition behaviour and not to decreased absence of parasitoid species or decreased parasitism rate. Furthermore, the parasitoids in the system may have only minor effects on the level of damage to the plant, nor directly by reducing the amount of food consumed by parasitised herbivores, nor by regulating the local population dynamics of the herbivore. Whether decreased seed set will affect local plant population dynamics is unclear, because *S. latifolia* is a perennial species and disturbance of soil and vegetation might be more important factors.

Although the results indicate that the herbivore is a better disperser than its three abundant parasitoids, only for one parasitoid species it was shown that fragmentation affected its presence at the scale of this study. No indications that habitat fragmentation might affect parasitoid behaviour, such as clutch size decisions or parasitism rates were found, although in large natural plant patches parasitism rate is higher. Maybe, all species in this system can cope with a higher degree of fragmentation than the current one. Although trophic interactions are locally affected by fragmentation, I expect that the current level of fragmentation does not threaten the persistence of any of the species in the study area.

Nederlandse samenvatting

Habitatfragmentatie

Elk organisme heeft een eigen habitat. Meestal wordt dit gedefinieerd als een gebied dat voorziet in de behoeften van die soort, zoals voedsel, nestelgelegenheid, klimatologische omstandigheden etc., waardoor individuen van die soort in dat gebied kunnen overleven en zich voortplanten. Voor plantenetende insekten wordt vaak het gebied waar de voedselplant voorkomt beschouwd als het habitat van die soort. Habitats kunnen van nature gefragmenteerd zijn door bijvoorbeeld bergketens en rivieren of doordat ze op eilanden voorkomen. Veel soorten leven dan ook in metapopulaties, die bestaan uit een aantal lokale subpopulaties die elk afzonderlijk een kans om uit te sterven hebben. Migratie tussen de habitatfragmenten zorgt ervoor dat er een evenwicht ontstaat tussen het uitsterven van subpopulaties en rekolonisatie, zodat de metapopulatie als geheel kan blijven voortbestaan.

Door menselijke aktiviteiten zoals wegenbouw, verstedelijking, ontbossing e.d. neemt landschapsversnippering sterk toe. Veel natuurlijke habitats raken daardoor steeds meer gefragmenteerd. Naast het directe effect van vermindering van de totale oppervlakte aan habitat voor een soort, worden er meestal drie effecten onderscheiden voor de overgebleven habitatfragmenten (Figuur 1.1): (a) de oppervlakte wordt kleiner, (b) de isolatie wordt groter, evenals (c) de hoeveelheid rand. Door habitatfragmentatie kunnen processen zoals migratie, kolonisatie en de lokale populatiedynamiek verstoord raken, waardoor het evenwicht van de gehele metapopulatie wordt verstoord en het voortbestaan van de soort in gevaar kan komen.

De laatste decennia is in Nederland het besef gegroeid dat landschaps-versnippering invloed kan hebben op het voortbestaan van soorten. Hieruit voortvloeiend zijn bijvoorbeeld de plannen van de regering voor de Ecologische Hoofdstructuur en de Groenblauwe Dooradering. Het doel van deze plannen is om de negatieve effecten van versnippering van natuurlijke habitats tegen te gaan door de verspreiding van soorten tussen habitatfragmenten weer mogelijk te maken.

De gevolgen van habitatfragmentatie

Zowel natuurlijke als door de mens veroorzaakte habitatfragmentatie kan verschillende effecten hebben op lokale populaties van soorten in de overgebleven habitatfragmenten. Door een verkleining van habitatoppervlakte worden de lokale populaties kleiner, waardoor ze kwetsbaarder worden voor willekeurige veranderingen in hun leefomgeving, de demografie of genetische eigenschappen. Een grotere isolatie en een verkleining van de omliggende habitatfragmenten verminderen de bereikbaarheid van een populatie waardoor het aantal immigranten kan afnemen. Niet alleen verkleint dit de kans op rekolonisatie van habitatfragmenten, maar lokale populaties worden ook minder aangevuld qua aantallen individuen en genetische variatie. Een vergroting van de hoeveelheid rand van een habitatfragment kan ervoor zorgen dat het omliggende milieu meer invloed heeft op de plek. Bovendien kan verspreidings- of eileggedrag van soorten anders zijn in randen dan in de kern van een habitat.

Al deze effecten kunnen lokale populaties van soorten kwetsbaarder maken voor uitsterven. Echter, niet alleen deze directe effecten van fragmentatie op soorten moeten in beschouwing worden genomen. Veel belangrijker, hele levensgemeenschappen kunnen worden verstoord omdat de meeste soorten elkaar beïnvloeden. Niet alleen is het voorkomen in een habitatfragment van organismen hoger in de voedselketen afhankelijk van het voorkomen van soorten lager in de voedselketen, ze kunnen ook juist de aantallen van die lagere soorten beïnvloeden. Voorbeelden zijn ziekteverwekkers die hun gastheer doden of planteneters die de plant aantasten.

De gevolgen voor plant-herbivoor-parasitoïd systemen

Dit proefschrift richt zich op de effecten van habitatfragmentatie op een tri-trofisch systeem, dat bestaat uit drie lagen in de voedselketen; een plant, een plantenetend insekt (herbivoor) en parasitoïden die de herbivoor aanvallen. Parasitoïden zijn insekten, voornamelijk sluipwespen en sluipvliegen, die hun eitjes op of in een ander insekt leggen (de gastheer) en waarvan de larven zich voeden met het insekt dat uiteindelijk sterft. Er zijn veel soorten sluipwespen die allemaal hun eigen levenswijze hebben. Zo zijn er interne parasitoïden waarvan de larven zich in de gastheer ontwikkelen en externe parasitoïden waarvan de larven de gastheer van buitenaf uitzuigen. Verder zijn er parasitoïden die het ei, de larve of zelfs de pop van een insekt gebruiken als voedselbron voor hun larven. Ook kan onderscheid worden gemaakt tussen solitaire en gregaire parasitoïden die respectievelijk één eitje of een groep eitjes afzetten op of in de gastheer. Vervolgens wordt nog onderscheid gemaakt tussen parasitoïden die de groei van de gastheer direct stoppen en diegenen die hun gastheer in leven laten en laten groeien tot de larven volgroeid zijn. Veel parasitoïden kunnen verschillende soorten insekten aanvallen (generalisten), maar anderen kunnen zich ontwikkelen op maar één of een paar gastheersoorten (specialisten).

Parasitoïden, kunnen op twee manieren zorgen dat planten minder schade ondervinden van plantenetende insekten. Ten eerste doden ze hun gastheren, zodat het aantal herbivoren in de volgende generatie vermindert. Ten tweede zorgen veel parasitoïden ervoor dat individuele gastheren minder eten als ze geparasiteerd zijn. Ze kunnen dus belangrijk zijn om schade aan planten te verminderen en worden dan ook ingezet in de biologische landbouw om schadelijke herbivoren te bestrijden. Uit verschillende studies is echter gebleken dat parasitoïden zich in overgebleven habitatfragmenten niet goed kunnen handhaven, waardoor de planten meer schade kunnen ondervinden van herbivoren, die over het algemeen minder gevoelig zijn voor habitatfragmentatie. Bovendien kan misschien ook het gedrag van parasotoïden veranderen, waardoor bijvoorbeeld de hoeveelheid eieren die per rups wordt gelegd, verandert.

Vraagstelling

De hoofdvraag van dit proefschrift is: heeft habitatfragmentatie invloed op de parasiteringsgraad van de herbivoor en heeft dit consequenties voor de hoeveelheid vraat aan de planten? Meer specifiek test ik of parasitoïden gevoeliger zijn dan herbivoren voor habitatfragmentatie en daardoor vaker afwezig zijn in habitatfragmenten en of dit leidt tot meer vraatschade aan de plant. In de onderstaande hoofdstukken van dit proefschrift komen verschillende deelvragen met betrekking tot de hoofdvraag aan bod:

Hoofdstuk 2

Welke soorten parasitoïden vallen de rups aan, wat is hun specifieke relatie met de rups en hoeveel rupsen worden geparasiteerd?

Hoofdstuk 3

Kan de belangrijkste parasitoïd in dit systeem de hoeveelheid vraat van geparasiteerde rupsen verminderen?

Hoofdstuk 4

Heeft habitatfragmentatie invloed op het voorkomen van het anjeruiltje en de belangrijkste sluipwespen in de habitatfragmenten?

Hoofdstuk 5

Heeft het niveau van habitatfragmentatie invloed op de hoeveelheid rupsen die geparasiteerd zijn en/of de hoeveelheid vraat in de fragmenten?

Hoofdstuk 6

Kan de leeftijd van de sluipwesp op het moment van parasitering, die misschien hoger is als een geïsoleerde plek wordt bereikt, de hoeveelheid eieren die een sluipwesp in een rups legt beïnvloeden?

Het modelsysteem:
de avondkoekoeksbloem, het anjeruiltje en haar parasitoïden

Gedurende 4 jaar heb ik de avondkoekoeksbloem, het anjeruiltje en de parasitoïden van het anjeruiltje bestudeerd. Van de avondkoekoeksbloem, *Silene latifolia*, bestaan zowel mannetjes- als vrouwtjesplanten (Figuren 1.2 A en B). De planten bloeien meestal ’s avond en ’s nachts. De witte bloemen geuren dan sterk en worden vooral bezocht door nachtvlinders, die voor de bestuiving zorgen. Eén van deze bezoekers is het anjeruiltje, *Hadena bicruris*. Deze nachtvlinder is niet alleen een bestuiver maar ook een herbivoor. De vrouwtjes leggen hun eitjes in de bloemen van de vrouwelijke plant (Figuur 1.2 C). De rupsen vreten vervolgens een klein gaatje in de nog onvolgroeide zaaddoos. Ze leven in de zich ontwikkelende zaaddoos tot ze alle zaden erin hebben opgegeten (Figuur 1.2 D). Vervolgens kruipen ze uit de zaaddoos en gaan op zoek naar andere zaaddozen (Figuur 1.2 E). Zo kunnen ze wel 5-7 zaaddozen leeg eten. De schade, veroorzaakt door de nakomelingen van één vlinderpaar, is daardoor veel groter dan het eventuele voordeel van bestuiving. Veel rupsen worden echter gedood door parasitoïden en misschien wordt de schade die de plant oploopt hierdoor beperkt.

De planten komen voor op plekken met verschillende niveau’s van fragmentatie langs de Rijn en de Waal. De rupsen van het anjeruiltje leven alleen van de avondkoekoeksbloem, dus kunnen deze plekken worden beschouwd als habitatfragmenten. Omdat de planten gemakkelijk te vinden zijn en we van alle plekken precies kunnen bepalen hoe groot (nl. het aantal aanwezige planten) en hoe geïsoleerd deze zijn, is dit systeem uitermate geschikt om de effecten van habitatfragmentatie te bestuderen.

Het parasitoïdencomplex op het anjeruiltje

In **hoofdstuk 2** beschrijf ik het gehele complex van parasitoïdensoorten die de rups van de nachtvlinder aanvallen, inclusief een aantal soortskenmerken, zoals legselgrootte (het aantal larven per rups), sekseverhouding en het voorkomen van overwintering. Gedurende drie jaren heb ik bijna 1800 rupsen verzameld van alle plekken met avondkoekoeksbloemen en uitgekweekt om de aanwezigheid van parasitoïden vast te stellen. Uiteindelijk bleek de helft van de rupsen geparasiteerd (Figuur 2.1). Tenminste 14 verschillende soorten parasitoïden werden gevonden, waarvan de specialist *Microplitis tristis,* de specialist *Eurylabus tristis* en de generalist *Bracon variator* de meest belangrijke zijn (Tabel 2.1). Deze drie sluipwespen ontwikkelen zich op verschillende manieren. De eerste ontwikkelt zich met meerdere larven in één rups (gregair), de tweede ontwikkelt zich solitair in de verpopte rups en de laatste ontwikkelt zich gregair op de buitenkant van een verdoofde rups. Alle parasitoïden vallen de grotere stadia van de rupsen aan, wanneer al veel schade aan de plant veroorzaakt is. Op één na, laten de meest voorkomende parasitoïden de rups nog voor een bepaalde tijd tijdens de ontwikkeling van hun larven leven en groeien, zodat ze geen sterke reductie veroorzaken in de schade die de rups aanricht. De conclusie is, dat parasitering er niet voor zorgt dat er minder schade aan de plant is. Er werden ook verscheidene soorten hyperparasitoïden gevonden, die zich ontwikkelen op de primaire sluipwespen (Figuur 2.7), maar deze hebben waarschijnlijk maar een beperkte invloed op hun aantallen. Net als bij veel andere gregaire sluipwespen ontwikkelen zich bij *M. tristis* en *B. variator* meer vrouwtjes dan mannetjes (Figuren 2.4 en 2.6). *B. variator* produceert echter bijna alleen maar legsels van dezelfde sekse (Figuur 2.5), waarschijnlijk een aanpassing om inteelt door paringen tussen broertjes en zusjes te voorkomen.

De gevolgen van gastheergrootte op de ontwikkeling van een sluipwesp en op de groei en de vraat door de gastheer

In **hoofdstuk 3** onderzoek ik in detail de relatie tussen de rups van het anjeruiltje en de meesbt voorkomende sluipwesp, *M. tristis*. Uit eerder onderzoek is gebleken dat veel gregaire interne parasitoïden die hun gastheer gedurende een bepaalde tijd laten leven en groeien, zoals *M. tristis,* voor een toename van de vraat door hun gastheer kunnen zorgen. In dit onderzoek werden rupsen van verschillende groottes en ontwikkelingsstadia aan de vrouwtjeswesp aangeboden. Ik heb het effect van parasitering gemeten op de hoeveelheid voedsel die de rups eet en op verschillende factoren die belangrijk zijn voor de nakomelingen van de wesp zoals legselgrootte, legselgewicht, overleving en ontwikkelingstijd. De resultaten laten zien dat de sluipwesp de ontwikkelingstijd en groei van de gastheer aanpast aan de grootte van de rups op het moment van eileg. In kleine gastheren werd de groei van de rups sterk geremd (Figuur 3.3), waardoor er meer competitie voor voedsel ontstond tussen de larven. En hoewel de ontwikkelingstijd van de larven ook langer werd (Figuur 3.1) en de groei van de rups sterker naarmate er meer larven in de rups aanwezig waren (Figuur 3.4), leidde dit er toe dat de sluipwespen in kleine gastheren kleiner bleven (Figuur 3.1) en een kleinere overlevingskans hadden (Figuur 3.2). De waargenomen beste ontwikkeling van de sluipwesp op de grotere rupsen komt overeen met de waarnemingen in het veld die laten zien dat alleen de grotere rupsen worden aangevallen door sluipwespen. In het experiment was de voedselconsumptie door geparasiteerde ruspen in alle stadia sterk verminderd (Figuur 3.4). Dit laat zien dat ook een gregaire wesp een vermindering van de hoeveelheid schade aan de plant kan veroorzaken.

De verspreidingspatronen van een herbivoor en drie van zijn sluipwespen in een gefragmenteerd landschap

In **hoofdstuk 4** worden de effecten van habitatfragmentatie op het voorkomen van het anjeruiltje en de drie meest belangrijke sluipwespen onderzocht. De verwachting was dat de sluipwespen vaker afwezig zouden zijn in kleine en afgelegen habitatfragmenten, omdat ze waarschijnlijk kwetsbaarder zijn en zich minder goed kunnen verspreiden. Gedurende drie jaar werd de aanwezigheid van het anjeruiltje in avondkoekoeksbloem-populaties langs de Waal en de Rijn met verschillende niveau's van fragmentatie (met als belangrijkste factoren de grootte, oftewel het aantal planten, en de mate van isolatie) vastgesteld en werden rupsen verzameld om het voorkomen van sluipwespen vast te stellen. Bovendien testte ik de hypothese dat sluipwespen minder goede verspreiders zijn dan het anjeruiltje door te kijken naar de kolonisatie van plekken met avondkoekoeksbloem die tot op een afstand van 2 km van bestaande habitatfragmenten waren gemaakt. De resultaten laten zien dat het anjeruiltje heel goed kan koloniseren, onafhankelijk van de afstand (Figuur 4.8), wat goed verklaart waarom het elk jaar kon worden gevonden in vrijwel alle onderzochte habitatfragmenten (Figuur 4.3). Ook de sluipwesp *M. tristis* lijkt een goede verspreider te zijn omdat zijn kolonisatie onafhankelijk was van de afstand (Figuur 4.8) en zijn aanwezigheid in de natuurlijke plantenpopulaties onafhankelijk was van de isolatie (Figuur 4.5). Dit is in tegenstelling met de resultaten voor de sluipwesp *E. tristis,* die wel negatief wordt beïnvloed in zijn aanwezigheid door de mate van isolatie (Figuur 4.5). Verder werd zijn aanwezigheid sterk beïnvloed door de grootte van het habitatfragment (Figuur 4.8), wat een mogelijke verklaring is voor het vrijwel ontbreken van kolonisatie van de kleine experimentele plekken. De generalistische *B. variator*, koloniseerde een gemiddeld aantal plekken, zijn verspreidingspatroon was niet gerelateerd aan de mate van isolatie en slechts zwak beïnvloed door de grootte van een plantenpopulatie. Aan het einde van het hoofdstuk bediscussieer ik verschillende eigenschappen van sluipwespensoorten, zoals populatiegrootte, niveau van gastheerspecialisatie en kolonisatievermogen, welke de verschillende verspreidingspatronen en reacties op fragmentatie zouden kunnen verklaren.

De gevolgen van fragmentatie op de hoeveelheid vraat en de parasiteringsgraad

In **hoofdstuk 5** worden de effecten van habitatfragmentatie op de hoeveelheid vraat door het anjeruiltje en de parasiteringsgraad bekeken. In de plantenpopulaties langs de Waal en de Rijn met verschillende niveau's van fragmentatie (grootte en isolatie) is het aantal aangetaste zaadkapsels en de hoeveelheid geparasiteerde ruspen gemeten. De analyses laten zien dat de vraat toeneemt met het kleiner en geïsoleerder worden van de plantenpopulaties (Figuren 5.2 en 5.3). Verminderingen van de parasiteringsgraad en het aantal soorten parasitoïden treden op als de habitatfragmenten kleiner worden, maar niet als ze geïsoleerder liggen (Figuren 5.4 en 5.5). Omdat het anjeruiltje een zeer goede verspreider is en de sluipwespen waarschijnlijk weinig invloed hebben op het aantal rupsen en de hoeveelheid voedselverbruik door de rups, is het onaannemelijk dat parasitering een grote invloed heeft op de lokale hoeveelheid vraat aan de plant. Een afname van parasitering kan dus niet verklaren waarom de vraat toeneemt in afgelegen en kleine plantenpopulaties. Een andere verklaring voor toegenomen vraatschade bij een hoger niveau van fragmentatie zou een verandering in eileggedrag van het anjeruiltje kunnen zijn. Ik heb dit getest door de hoeveelheid eieren, de hoeveelheid vraat en de hoeveelheid parasitering te meten in grote (met 256 planten) en kleine (met 16 planten) experimentele plantenveldjes. De hoeveelheid gelegde eieren was inderdaad relatief groter in kleine veldjes (Figuur 4.7 A) en aan de randen van de grote (Figuur 4.8 A). Ook de hoeveelheid vraat was verhoogd (Figuren 4.7 C & 4.8 B), maar er was geen effect van veldgrootte of afstand tot de rand op de parasiteringsgraad (Figuren 4.7 D & 4.8 C). De resultaten in dit hoofdstuk suggereren dat door de drie effecten van habitatfragmentatie (kleiner worden, geïsoleerder raken, of naar verhouding meer rand krijgen) de hoeveelheid vraatschade aan planten in habitatfragmenten hoger kan worden, niet omdat parasitoïden ontbreken, maar omdat het anjeruiltje zijn eileggedrag verandert.

Leeftijdsafhankelijke legselgrootte in een sluipwesp

In **hoofdstuk 6** heb ik gekeken wat het effect van leeftijd is op het aantal eieren dat een sluipwesp legt. Men kan zich voorstellen dat het door habitatfragmentatie langer kan duren voordat een sluipwesp een rups tegenkomt. Nu zijn er allerlei modellen ontwikkeld die voorspellen wat voor een vrouwtjessluipwesp het gunstigste is om te doen om zoveel mogelijk nakomelingen te krijgen. Als de wesp ouder wordt en weinig gastheren tegenkomt, neemt de kans toe dat zij niet al haar eieren kwijt zal raken. Daarom moet de wesp zoveel mogelijk eieren in de rups leggen (tot een bepaald maximum) die zij wel tegenkomt. Als de wesp echter al op jonge leeftijd een rups tegenkomt, is het beter om wat minder eieren te leggen, omdat de kans groot is dat zij ook nog andere rupsen tegenkomt. Door haar eieren dan te verdelen over meer rupsen verkleint de sluipwesp de kans dat ze allemaal samen met de rups, door bijvoorbeeld een vogel, worden opgegeten. Maar aan de andere kant is het natuurlijk zo, dat hoe ouder de vrouwtjeswesp wordt, hoe groter de kans is dat zij zelf dood gaat. Een wesp zou dus als zij vaak rupsen tegenkomt, in het begin meer eieren moeten leggen dan op latere leeftijd. Ondanks alle voorspellende modellen zijn er maar weinig studies die deze voorspellingen ook experimenteel getest hebben. In dit hoofdstuk wordt de wesp *M. tristis* gebruikt om de modellen te testen. Ik heb wespen op verschillende leeftijden rupsen van het anjeruiltje gegeven en vervolgens gekeken hoeveel eieren ze hadden gelegd. Daarbij is onderscheid gemaakt tussen wespen die ouder werden, maar wel elke dag een rups kregen en wespen die ouder werden maar geen rups ontvingen tot ze een bepaalde leeftijd hadden bereikt. Uit de resultaten blijkt dat tot een leeftijd van 2 weken de verschillende behandelingen en de leeftijd geen invloed hebben op de hoeveelheid eieren (Figuren 6.1, 6.2 en 6.3), in tegenstelling tot wat de modellen voorspelden. Pas op een waarschijnlijk onnatuurlijke leeftijd van 2 weken is een duidelijke afname te zien in de hoeveelheid eieren, ook als de wespen de eerste rups pas op hoge leeftijd ontvingen. Waarschijnlijk zijn de modellen niet geschikt voor het voorspellen van het gedrag van de onderzochte wesp. Daar zijn verschillende argumenten voor. Bijvoorbeeld dat de wespen genoeg eieren kunnen aanmaken (Figuur 6.7) voor de hoeveelheid rupsen die ze normalerwijze in het veld kunnen tegenkomen en dus niet zuinig hoeven te doen. Ook kan het zijn dat de wespen zijn aangepast aan een bepaalde hoeveelheid rupsen en eenvoudigweg geen mechanisme hebben om het aantal eieren te kunnen aanpassen.

Conclusies

Wat kunnen kan men concluderen uit al deze afzonderlijke onderzoeken met betrekking tot habitatfragmentatie?

Ten eerste was verwacht dat sterk gefragmenteerde populaties van de avondkoekoeksbloem een kleinere kans hadden op de aanwezigheid van het anjeruiltje en, in sterkere mate, op die van de parasitoïden. Dit blijkt maar ten dele waar. De rups is in vrijwel elke plek aanwezig. De verspreiding van het anjeruiltje is dus erg goed. Voor de sluipwespen zijn sterkere effecten van fragmentatie op hun aanwezigheid gevonden. Inderdaad komen er minder parasitoïden voor in sterker gefragmenteerde plantenpopulaties, maar dit was niet gerelateerd aan isolatie, alleen aan de hoeveelheid planten. Slechts één sluipwesp, *E. tristis*, bleek echt last te hebben van isolatie. De andere wespen kunnen zich erg goed verspreiden of ze zijn generalisten die ook rupsen in andere habitats kunnen parasiteren. *M. tristis*, de meest voorkomende specialist, blijkt zelfs vrij gemakkelijk 2 kilometer af te leggen. Blijkbaar kunnen sommige parasitoïden zich wel degelijk goed verspreiden en hebben ze dus weinig last van toenemende isolatie.

Uit de analyse van de levenswijzen van de verschillende soorten sluipwespen kunnen we opmaken dat de hoeveelheid vraat maar weinig afneemt ten gevolge van de parasitoïden. Ook al vermindert de meest voorkomende wesp de hoeveelheid gebruikt voedsel per rups (hoofdstuk 3), de kanttekening moet worden gemaakt dat er in dat experiment veel minder eieren per rups waren afgezet dan in de natuur gebeurt en waarschijnlijk groeit en eet de rups meer naarmate er zich meer larven in ontwikkelen. Ook is gebleken dat de hoeveelheid eieren die door de wesp gelegd wordt waarschijnlijk niet verandert als gevolg van veranderingen in leeftijd van de wesp. Verder is de invloed op de hoeveelheid rupsen in de volgende generatie in een plantenpopulatie waarschijnlijk beperkt omdat de vlinder zich zo goed kan verspreiden en zo veel eieren kan leggen. Daardoor heeft sterfte als gevolg van parasitoïden waarschijnlijk weinig invloed op de hoeveelheid eieren die gelegd worden door de vlinders in een plantenpopulatie. De conclusie moet dan ook zijn dat de gevonden negatieve effecten van habitatfragmentatie (en dan vooral door een verkleining van de fragmentgrootte) op de hoeveelheid parasitoïden-soorten en op de parasiteringsgraad, geen grote consequenties hebben voor de hoeveelheid vraatschade aan de planten.

Toch zien we een toename van vraat bij een toenemende niveau van fragmentatie. Waarschijnlijk is dit het gevolg van een verandering in eileggedrag van de vlinder. In kleine en geïsoleerde plantenplekken worden per plant meer eieren gelegd, misschien door een toename in de hoeveelheid rand. Of deze toegenomen vraat een negatieve invloed heeft op de plantenpopulaties heb ik niet onderzocht. Het lijkt erop dat waargenomen groei of inkrimping van de plantenpopulaties over de drie jaar van mijn onderzoek niets met fragmentatie te maken heeft, maar veel eerder met verstoring of verandering van de plaatselijke vegetatie. Misschien is de hoeveelheid overlevende zaden wel minder belangrijk dan de aanwezigheid van goede kiemplekken.

De goede verspreiding van de meeste soorten in dit systeem geeft wel aan dat het huidige fragmentatieniveau niet echt leidt tot problemen bij de onderzochte soorten. Misschien zijn deze organismen wel aangepast aan een hoger niveau van landschapsversnippering, want er zijn aanwijzingen dat de avondkoekoeksbloem nu veel meer voorkomt in Nederland dan vroeger.

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Curriculum vitae

Jelmer Elzinga was born on the 17th of June 1976 as first son of G. Elzinga and T. Elzinga-van der Brug in Leeuwarden. After completing his education at the Christelijk Gymnasium in Leeuwarden he started the study of biology at the University of Groningen in 1994. For his specialisation in ecology from 1997 onwards, he carried out three undergraduate research projects. The first was a greenhouse study on the effects of microbial mats in calcareous wet dune slacks on vegetation succession, supervised by Ab Grootjans and Hans van Gemerden. For the second project under supervision of Jan Bakker, he did fieldwork in grasslands near the Drentse Aa and analysed vegetation data of 25 years to evaluate the development of the vegetation towards species rich grassland with different mowing regimes. In 1999 he started his final undergraduate project on the seed dispersal strategies of the red acouchies in the tropical forest. Under supervision of Patrick Jansen and Frans Bongers and together with Martijn Bartholomeus he stayed 4 months in the tropical rain forest of French Guyana.

In April 2000 Jelmer started his PhD project in the Department of Plant Population Biology at the Centre for Terrestrial Ecology from the Netherlands Institute of Ecology (NIOO-KNAW) in Heteren. With the cooperation of Prof. Dr. Jos van Damme, Dr. Arjen Biere, Dr. Jeffrey Harvey and others, he studied how trophic interactions between plants, herbivores and parasitoids can be affected by habitat fragmentation with *Silene latifolia*, *Hadena bicruris* and its parasitoids as an example system. He combined data gathered from field work along the rivers Rijn and Waal in Gelderland, with experiments in the experimental garden and the laboratory in Heteren. The result of this work is presented in this thesis. During the PhD project he has been involved in several other studies concerning the enemies of *S. latifolia* and parasitoid ecology.

In December 2004 he started working as a postdoctoral research associate in the group of Giorgina Bernasconi at the Department of Ecology and Evolution of the University of Lausanne, Switzerland, to continue his work on *S. latifolia* from a more evolutionary perspective.

Publication list

Thesis work

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