Interactions between natural enemies and the dioecious herb *Silene dioica*

Viktoria Pettersson

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Department of Ecology and Environmental Science
Umeå University
SE-901 87 Umeå
Sweden

Akademisk avhandling

Som med vederbörligt tillstånd av Rektorsämbetet vid Umeå universitet för avläggande av filosofie doktorsexamen i ekologi kommer att offentligen försvaras lördagen den 25 april, kl 10.00 i (KB3B1), KBC. Avhandlingen kommer att försvaras på engelska.

Examinator: Professor Lars Ericson, Umeå Universitet

Fakultetsopponent: Docent Pia Mutikainen, Institute of Intergrative Biology, Swiss Federal Institute of Technology, Zürich, Schweiz.
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About 6% of all angiosperms are dioecious. This separation of sexual function to male and female individuals, and the fundamentally different patterns of reproductive resource allocation that follows that separation, are thought to have important ecological and evolutionary consequences for plant enemy interactions. I have studied whether intersexual differences in susceptibility to natural enemies can be explained by intersexual differences in resource allocation. In cases when sexual dimorphic traits for the target resource of a particular enemy I expected the enemy to select the best resource.

The study system is the perennial dioecious herb, *Silene dioica* (Caryophyllaceae) and three of its specialist natural enemies, two insect herbivores the fly *Delia criniventris* (Anthomyiidae) and the twirler moth *Caryocolum visciariella* (Gelechiidae) and one systemic anther smut fungus *Microbotryum violaceum*. All three share the same food resource, the floral stems, of their host plant. I studied the interaction on nine islands in a rising Bothnan archipelago over seven consecutive years.

Both herbivores attacked female plants more than male plants (*D. criniventris*, 32.8% females, 30.7% males; *C. visciariella*, 4% females, 2% males). This attack pattern was consistent over years and islands and also correlated with a number of sexually dimorphic traits suggesting that females offer the better resource. Herbivore attack had no effect on plant survival but a significant effect on re-flowering the following year. Non-attacked females had an estimated mean re-flowering rate of 30.2%, and non-attacked males of 31%. Herbivore-attacked females had an estimated re-flowering rate of 46% compared with 38.4% for males. Females showed a stronger compensatory response to attack and tended to re-flower more often than males. Attack rates differed markedly in the different stages of primary succession. They were consistently higher in the youngest zone and decreased in parallel to progressing succession. This zonal pattern of decreasing attack rates correlated with several plant attributes, a decrease in plant size and nitrogen content, and an increased content of secondary compounds, but not to host plant density. We failed to come up with a simple explanation for the spatial structure with chronic high attack rates in the younger zones. However, the consistent patterns in attack rate suggest that a suite of abiotic and biotic factors interact and reinforce the strength and direction of selection.

In general females were more frequently diseased by the anther smut *Microbotryum violaceum* than males with two exceptions. Disease frequencies were male biased on islands with low disease levels and in one of the seven study years. The change in disease frequencies from male to female bias confirm earlier studies suggesting that the relative contribution of the two components of infection risk, disease encounter and per contact infection probability can vary with population disease level. The change in the proportions of diseased males and females that was observed in one of the study years, followed a year of extreme weather conditions (prolonged drought). Both sexes showed a similar decline in flowering but diseased females decreased more than diseased males. This difference in response can be explained if considering that disease is more resource demanding in females than in males. Except for resources needed for mycelial growth and spore production, in females resources are also needed to restructure their sex expression and produce anthers.

My study shows (i) that in dioecious species traits that are sexually dimorphic are of great importance for understanding the outcome of interactions with natural enemies, (ii) that the strengths and directions of enemy-host plant interactions are strongly shaped by both abiotic and biotic conditions.

**KEYWORDS:** *Silene dioica, Delia criniventris, Caryocolum visciariella, Microbotryum violaceum, plant-herbivore interactions, plant-pathogen interactions, sex bias, sex ratio, successional gradients, fitness effects, Skeppsvik Archipelago*
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Umeå University
SE-901 87 Umeå
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List of Papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

I  Pettersson V., Gardfjell H., Witzell J., Ericson L. & Giles B.E. Sex-biased herbivory in *Silene dioica*. Which sex is the better resource? Manuscript

II Pettersson V., Gardfjell H., Ericson L. & Giles B.E. Male and female responses to florivory in the perennial herb *Silene dioica*. Manuscript

III Pettersson V., Gardfjell H., Witzell J., Ericson L. & Giles B.E. Rates of insect herbivory on *Silene dioica* change across primary successional zones. Manuscript

IV Pettersson V., Gardfjell H., Ericson L. & Giles B.E. Which sex is most sensitive to a sexually transmitted disease? A case study of the *Microbotryum violaceum-Silene dioica* association. Manuscript
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Introduction

Natural enemies are always present and consist of a variety of organisms and life forms including pathogens (viruses, bacteria, protozoans and fungi), parasites (helminths and arthropods), parasitoids (e.g. arthropods) and predators (most nonparasitic animal taxa) (e.g., Shea et al. 2000). Similarly, hosts occur at all levels of biological organization and consists of plants, invertebrates, vertebrates; and even pathogens themselves can be hosts (Shea et al. 2000). This thesis will specifically focus on plant-enemy interactions and particularly the interactions between plants and their herbivores and fungal pathogens.

Plant-enemy interaction

Herbivores remove on average one fifth of all terrestrial plant biomass and about half of all aquatic biomass (Cyr and Pace 1993). The desire to understand patterns of herbivore damage on plants has lead to a rich literature aim to increase our understanding of when and why plants are attacked. The numbers, sizes and chemical composition of plants and their distribution in space may affect the births, deaths and dispersal rates of their herbivores (Crawley 1983). Herbivore feeding, may influence the probabilities of establishment, growth and seed set of the plants upon which they feed (Crawley 1983). Herbivory can affect the probability of plant survival, growth rates and reproduction (Crawley 1983; Hendrix 1988; Marquis 1992). Normally the mortality of mature plants is not affected by herbivores (Crawley 1983; Strauss and Zangerl 2002). However seedlings are more vulnerable and can therefore experience higher mortality due to herbivory than mature plants (Hendrix 1988; Strauss and Zangerl 2002). Herbivores will affect plant growth rate in different ways depending on what kind of plant part they utilise: leaf herbivory will reduce the photosynthetic area, sapsuckers and seed eaters can alter the carbohydrate balance, rot feeders and xylem suckers can interfere with water and nutrient uptake and shoot and stem bores can weaken the physical structure of the plant (Crawley 1983). Plant reproduction can be reduced by herbivores in several ways; the most obvious way is by preventing plants to enter the reproductive stage or by shortening the life span of individual plants (Hendrix 1988). Folivory can reduce flower, fruit and seed set (Hendrix 1988) but can also reduce pollen number and
pollen size (Lehtilä and Strauss 1999). Herbivory that is not limited to vegetative tissue i.e. damage on flowers or reproductive tissue can also have both direct and indirect effects on plant fitness (McCAll 2007 and references therein). If pistils, ovaries or ovules are eaten there will be a direct effect on female reproductive function (Zammit and Hood 1986; Schemske and Horvitz 1988; Wallace and Odowd 1989; Bertness and Shumway 1992; Englishloeb and Karban 1992; Pellmyr and Huth 1994). Male reproductive function can directly be diminished by anther or pollen consumption (Krupnick and Weis 1999). Plants may also experience indirect effects such as lower pollination service (Karban and Strauss 1993; Cunningham 1995; Krupnick et al. 1999) caused by differences in floral appearance and reduction in floral display (Krupnick and Weis 1999). All these effects of florivory will lead to the production of fewer and/or smaller and lower siring success. All of this demonstrates that herbivory influence individual plant fitness negatively and may further have important influence also on different demographic parameters of plant populations.

Fungal pathogens may affect host plant fitness in different ways depending on what kind of disease a plant is affected by. Soil-borne pathogen (e.g. root rots) have detrimental effects on the survival of host plants, cankers and wilts (ex Dutch elm disease and chestnut blight) have negative effects on growth and survival, foliar pathogens (ex. rust and powdery mildews) may affect host plant survival, reproduction, growth and competitive ability. Non-systemic floral diseases have only been shown to negatively affect plant reproduction whereas systemic fungal diseases (e.g., rusts and smuts) can have both positive and negative effects on plant survival, reproduction, growth, competitive ability and susceptibility to other pest (reviewed in Jarosz and Davelos 1995). Fungal parasites do not always have negative effect on host plant fitness but may have large effect on plant’s ability to survive, reproduce, grow or defend itself against other parasite and herbivores (Jarosz and Davelos 1995).

**Plant life history and natural enemies**

Not only do plant enemies influence individual plant fitness they may also have direct impact on plant population dynamics. The impact of an enemy on its host plant depends on both the severity and the timing
of the attack. The manners in which a plant respond or compensate for such an enemy attack depend of life history strategies and sexual system. For instance annual plants have to respond and compensate directly for a loss of fecundity or they will suffer a direct loss of total life-time fitness (e.g., Maron 1998) while perennial plants can compensate for herbivory across years since they have the ability to accumulate resources over several years and can defer seed production until conditions are more favourable (Hendrix 1988).

The sexual system of the host plant can also affect the interaction between plants and natural enemies. Intersexual dimorphisms in life history in dioecious plants, with male and female function on separate plants, have resulted in different resource allocation to growth, reproduction and survival in males and females. Such differences can be caused by differences in resources needed for pollen and seed production (Delph 1999; Obeso 2002). In general, it is thought that production of seeds is more resource demanding than production of pollen and females are thus expected to allocate more resources to reproduction than males (Putwain and Harper 1972; Freeman et al. 1976; Lloyd and Webb 1977; Grant and Mitton 1979; Hancock and Bringhurst 1980; Wallace and Rundel 1980; Barrett and Helenurm 1981; Lovett Doust et al. 1987; Allen and Antos 1988; Ågren 1988; Delph 1999; Obeso 2002). Delph (1999) reviewed patterns of sexual dimorphism in life history traits and showed that the total biomass invested in reproduction was higher for females in 31 species, equal in two species where as no species showed higher allocation in males. In species where females invest more in reproduction females were generally older and larger than males at the time of first reproduction. Males on the other hand, flowered more frequently in 63 % of the species and also invested more in growth (69% of the species) compared to females. Males also have equal or longer life spans in 73% of the species where as in 19% of the species females either grow more and/or live longer than males (Delph 1999). Theory on the cost of reproduction suggest that there is a trade-off between resource allocation and either vegetative growth or reproduction (e.g., Obeso 2002). Sex differences in growth may be connected to plant palatability to herbivores and according to Herms and Mattson (1992) there is an additional trade-off between growth and defence i.e. plants that invest more in growth (generally males) may invest less in defence. Differences in energy expenditure between the sexes have
been shown to influence attack rates by herbivores and pathogens (Boecklen and Hoffman 1993; Boecklen et al. 1994; Ågren et al. 1999; Cornelissen and Stilling 2005). In a meta-analysis of 33 studies Cornelissen and Stilling (2005) concluded that male plants in general exhibited higher abundances of herbivores and suffered significantly more damaged than female plants. Ågren et al. (1999) showed that males were preferred by herbivores in 17 out of 21 species. In two species (Artemisia vesicaria and Rumex acetosella) females plants were preferred by some herbivores at some sites and in three species no difference between the sexes could be found (Ågren et al. 1999). Differences in herbivory on male and female plants are often more pronounced for damage to flowers, buds and/or seeds but also present in cases where leaves are attacked (Ashman 2002). Plant pathogens, on the other hand, show a more diverse response to plant sex (Ågren et al. 1999). For some pathogens disease is female biased, as demonstrated by studies of rusts (Lovett Doust and Cavers 1982; Åhman 1997) smuts. (Lee 1981; Alexander and Antonovics 1988; Shykoff et al. 1996; Kaltz and Shykoff 2001), for others, disease is male-biased, as demonstrated by an anamorphic red-spot disease (Ågren 1987) and smuts (Thrall and Jarosz 1994a; Biere and Antonovics 1996; Biere and Honders 1998), and yet for others no differences have been reported, as observed for a rust (Åhman 1997) and for smuts (Kirby 1988; Soldaat et al. 1997; Biere and Honders 1998). Depending on how pathogens are transmitted there are several possible explanations for why males and females differ in their susceptibility to parasites (e.g., Ågren et al., 1999). Wind-dispersed pathogens might react to differences in phenology of leaf production, leaf longevity and leaf area exposed (Burdon 1987; Dix and Webster 1995). Vector-borne pathogens on the other hand, generally respond to sex differences in floral display, and the sex with larger floral display size (generally males) will have a higher susceptibility to pollinator-transmitted pathogens (Alexander 1992). Frugivore transmitted pathogens have shown the opposite pattern, with females being more susceptible to parasites (Gehring and Whitham 1992).

Resource allocation to different life-history traits may also depend on what resources are available. The availability of resources (nutrients, water and light) may vary across successional gradients with important implications for plant growth and plant chemistry as well as enemy response. Plants growing in different successional stages have
been shown to have different strategies and therefore their interaction with herbivores may vary. Early successional plant species are often selected for rapid growth and early maturation and as they generally grow under resource rich conditions this may increase their palatability to herbivores as they invest comparatively little in defensive structure or in secondary chemistry (Cates and Orians 1975; Feeny 1976; Rhoads and Cates 1976; Bryant et al. 1983; Southwood et al. 1986; Coley 1988). Plants in intermediate successional stages are selected to have traits that allow them to have high rates of resource capture and therefore are able to compensate for loss of tissue to herbivores (Mooney 1972; Grime 1977; Bazzaz 1979; Chapin 1980; Coley 1983; McNaughton 1983; Grime et al. 1997). Plants in old/late successional communities experience poor nutrient conditions and these plants tend to grow more slowly, invest more in structural and chemical defense and as a consequence are expected to be less palatable to herbivores (Close and McArthur 2002). A wealth of studies has addressed the nature and extent of chemical defense in plants and whether it correlates with the successional status of the plant community. The main message of these studies is that herbivores prefer early successional plants to later ones in both terrestrial and aquatic systems (Grime et al. 1968; 1996, Cates and Orians 1975; Coley 1983; Godfray 1985; Southwood et al. 1986; Coley 1988). More rarely have pathogen disease levels been studied under different environmental conditions. However, moisture (Paul 1990), shading (Jarosz and Burdon 1988; Wennström and Ericson 1990) and nutrient levels (Paul and Ayres 1986; Wennström and Ericson 1992) have been found to influence the degree of susceptibility among individual plants and plant populations. In two of the few available studies of fungal pathogens Carlsson et al. (1990) demonstrated that disease incidence of the anther-smut fungus *Microbotryum violaceum* on *Silene dioica* varied considerable in populations of different age and Carlsson-Granér (1997) concluded that young and old successional zones had low proportion of *M. violaceum* disease whereas the intermediate successional parts of the population had high proportions of infection.
**Pathogen-herbivore interaction**

Herbivores may also affect the influence of fungal pathogens on local host population dynamics by altering the probability of fungal transmission or disease spread. Disease incidence of a foliar rust fungus on *Trifolium pratense* was lower (about ¼) in deer-grazed control plots compared to enclosures (Bowers and Sacchi 1991). The same effect was found for the interaction between the host plant *Tridentalis europaea* and the smut fungus *Urocystis trientalis*; when voles were excluded that resulted in higher disease incidence and an increase in host densities (Ericson and Wennström 1997). In addition (Wennström and Ericson 1991) found a ten-fold lower incidence of the host-sterilizing, systemic rust *Puccinia pulsatillae* in grazed than in ungrazed sites of *Pulsatilla pratensis*.

**Objectives of the thesis**

The objectives of this thesis were to answer the following questions:

- Do *Silene dioica* experience sexual dimorphism in plant characters important for natural enemies? If so do the florivores *Caryocolum viscariella* and *Delia criniventris* respond by attacking female or male plants of *S. dioica* differently? *(Paper I)*
- How do of male and female *S. dioica* plants respond to florivory? *(Paper II)*
- Do we detect changes in *C. viscariella* and *D. criniventris* herbivory on *S. dioica* across primary successional zones? *(Paper III)*
- Is there intersexual difference between male and female *S. dioica* plants in *Microbotryum violaceum* disease? Are there differences in *M. violaceum* disease over a successional gradient? *(Paper IV)*
- Can we find evidence of positive or negative interactions between *M. violaceum* and mammal herbivores? *(Pathogen-herbivore interaction)*

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Material and methods

Study site

This study was conducted in the Skeppsvik Archipelago in the Gulf of Bothnia, Västerbotten province, Sweden (63°44'-48'N, 20°34'-40'). New islands are continually created by isostatic land uplift (0.9 cm/yr). As a consequence of this each island, and part of an island, goes through a primary succession dominated sequentially by grasses, shrubs, deciduous trees and coniferous trees (Ericson and Wallentinus 1979). Islands in the archipelago differ in age and a successional gradient also occur across islands. There is also a soil nitrogen gradient within the islands where the nitrogen varies from its lowest value by the shoreline, the highest value in young zones, after which of nitrogen content decline in the intermediate zone and continue to decrease when reaching old zones (Havas 1967) (Figure 1).

For paper III and IV, we defined the successional stages young (Y), intermediate (I) and old (O) depending upon their position along the studied chronosequence (Figure 1).

Figure 1. Illustration of vegetation types characteristic of 120-150 year old islands in the Skeppsviks Archipelago. Young, intermediate and old refer to the three zones used to describe the primary successional gradient.
Study system

The study system consists of the interaction between the host plant *Silene dioica* (L.) Clair (Caryophyllaceae) and three associated natural enemies: the two florivores *Caryocolum viscariella* (Stainton) and *Delia criniventris* (Anthomyiidae) (Zetterstedt) and the pathogen *Microbotryum violaceum* ((Ustilaginales; synonym *Ustilago violacea*) (Brandenburger and Schwinn) G. Deml. & Oberw.).

**The host plant**

*Silene dioica* (L.) Clair (Caryophyllaceae) is a dioecious, insect pollinated, perennial herb found in disturbed habitats throughout most of northern and central Europe (Jonsell 2001). In the study area, the Skeppsvik archipelago, *S. dioica* belongs to a middle successional zone characterised by the presence of deciduous trees (Giles and Goudet 1997b; Giles and Goudet 1997a). *S. dioica* is dioecious with a chromosomal sex–determination system were females are XX and males are XY (Westergaard 1958). Plants grow from a winter green basal rosette from which flowering shoots are produced. Established plants live for 5 – 12 years, flowering first when 2-3 years old; males reach reproductive maturity earlier than females and mature individuals do not necessarily flower each year (Carlsson-Granér et al. 1998; Giles et al. 2006). Levels of vegetative spread are low and the dynamics of population growth depend on sexual reproduction (Giles and Goudet 1997b). Flowering starts at the end of May and peaks in late June/early July. Males start to flower earlier in the season than females and flower for longer periods. Females produce capsules filled with seeds which ripe in August and seeds disperse passively by gravitation. The seeds germinate in spring, germination success varies between 20-70% (B. E. Giles unpublished data) and seedling mortality is about 80% (unpublished data).

**The florivores**

The microlepidopteran *Caryocolum viscariella* (Stainton) is a member of the family Gelechiidae. The Gelechiidae are a large family of many genera and species and where host specialisation is rampant (Huemer 1988). The genus *Caryocolum* consists of 63 species, each of which is
more or less specialised on one (sometimes two) species within the Caryophyllaceae (Huemer 1988). The larvae of *C. viscariella* attack the reproductive stems of *S. dioica* just as they begin to elongate, spinning together the upper leaves. They eat the developing buds and continue into the stem and the result is that the stem dies from the top down. We suspect that *C. viscariella* has a univoltine lifecycle in the study area and that the species overwinter as either egg or the first instar larva since first we have been able to hatch collected larvae in the same growing season and adults have been observed in end of July and beginning of August (Lars Ericson per.obs.). It is unlikely that adults are the overwintering stage since experimental plants that have been grown in a bench yard but left outdoors for vernalization have shown *C. viscariella* attack after they have been dug out of the snow and brought into the greenhouse for flowering. Thus egg or first instar larva had to been present prior to winter.

The fly *Delia criniventris* (Anthomyiidae) (Zetterstedt). Not much is known about the life history of most of the *Delia* species, however, the majority seem to have phytophagous larvae (Griffiths 1991). The larvae of *D. criniventris* are probably monophagous in our system and Kaltenbach (1874) reports nothing besides *S. dioica* as a food source for this species. The larvae of *D. criniventris* attack and eat the stem and the root system or meristem of *S. dioica* and consequently cause substantial damage. The larvae start in the above ground stem and the stem is killed from the bottom and upwards. The larvae can also move from one stem to the other via the meristem and will pupate at the beginning of the tap root (Kaltenbach 1874, V. Pettersson pers. obs.). We do not know much about the life cycle of *D. criniventris* however, we suspect it to be univoltine with a diapausing egg. This conclusion is based on three observations. First we have observed pupae in the middle of July in the studied area and also we have been able to hatch pupae in the laboratory the same growing season as they were collected. Second adults have been observed on the inflorescences of *Filipendula ulmaria* during July and early August on our studied islands (L. Ericson pers. obs.). We assume that they are foraging on *F. ulmaria* because most Dipteran are dependent of adult feeding for egg maturation and it has been observed that that adult flies from the Anthomyiidae family feed on exposed fluids including floral and extra floral nectar and pollen (Wäckers et al. 2007). Third we have observed infested *D. criniventris* stems in greenhouse population of *S. dioica*
early in the season as for *C. viscariella* and this observation is not in line with overwintering adults. Although we cannot exclude the first instar larvae as the diapausing stage based on our observations however, a study by Johansen (1990) on the biology of natural populations of *Delia fabricii* in northern Norway with the similar short growing season as our studied area, the females lay eggs in August/September on the shoots of their host or on the soil adjacent the host plants and the egg is the diapausing stage. This information together with our observation makes us conclude that *D. criniventris* have a univoltine lifecycle with an over wintering egg.

*The pathogen*

*Microbotryum violaceum* ((Ustilaginales; synonym *Ustilago violacea*) (Brandenburger and Schwinn) G. Deml. & Oberw.) is a systemic perennial anther-smut fungus and if it successfully manages to establish in the basal meristem of a host, the host will become systematically infected (Hassan and MacDonald, 1971) and therefore become permanently sterile. It is mostly spread by pollinators carrying dispersal spores to healthy plants (Jennersten 1983; Alexander and Antonovics 1988; Jennersten 1988; Thrall et al. 1993) in our system bumble bees (*Bombus spp.*) are the main vector (Giles et al. 2006). Floral infection is the most common path although seedlings growing adjacent to diseased plants may become infected through passive spore dispersal (Baker 1947b; Hassan and Macdonald 1971; Alexander and Antonovics 1988; Roche et al. 1995). There is no vertical transmission of the pathogen via seeds (Baker 1947b; Spencer and White 1951) since the fungus causes abortion of the ovaries and therefore does not enter the seeds (e.g., Thrall et al. 1993). The smut alters the structures of female sex organs (the ovary becomes rudimental and sterile) so both female and male flowers produce anthers filled with fungal teliospores (Baker 1947a). However, it is still possible to distinguish between infected flowering males and females by feeling for the presence of a rudimentary ovary.

*Survey*

Screening was carried out across successional gradients on nine island located in different positions within the archipelago (Figure 2). A total of 222 permanently marked 5x5 m plots were established on these
islands enabling the same areas to be surveyed each year and with considerable replication of each successional stage within the archipelago. Between 2000-2006, plots were screened at peak flowering to assess the numbers of flowering individuals, densities of male and female flowering plants and rates of C. viscariella and D. criniventeris attack and M. violaceum disease. To assess the number of floral stems available to insect herbivores, all stems were counted per individual in 2005 and 2006. Attacks by C. viscariella and D. criniventeris were not recorded separately in survey from 2000-2003, but it is possible to analyze attack patterns for each herbivore in data collected from 2004-2006. Where possible, all 5x5 m plots contain

Figure 2. The Skeppsvik Archipelago. The nine study islands are numbered in order of increasing age.
one or more permanent 1 m² demographic plots. In these plots, individuals older than one year is marked with a plastic stick with an individual number that is inserted into the ground at the base of the plant. To allow us to follow the fates and transitions of individuals between different life stages, individuals were assigned each year to one of the six stages (1) seedling, (2) small pre-reproductive vegetative; established individuals 2-3 years old, (3) large pre-reproductive vegetative; established individuals 3-4 years old; (4) flowering reproductive individuals, (5) post-reproductive vegetative; a vegetative state in individuals known to have flowered, (6) dead. Each year data were collected on stage, sex, *C. viscariella* and *D. criniventris* attack and mortality for each plant. These data are available from 1998-2005. In 2004 and 2005 the basal leaf rosette diameter, stem and capsule numbers were also recorded. In 2000 and 2001 we built enclosures to exclude mountain hare (*Lepus timidus*) and roe-deer (*Capreolus capreolus*). The enclosures were randomly assigned to the 5x5 m plots with a minimum of one per island.

**Chemical analyses**

In paper I and III we evaluated plant quality for the florivores by we measuring nitrogen and soluble phenolics in *S. dioica* leaves. Nitrogen content was measured because nutrient content in plants have been shown to influence insect herbivore survival and reproduction (Bernays and Chapman 1994; Awmack and Leather 2002) also herbivores often prefer plants and plants part with higher nitrogen content (Mattson 1980; Price 1991). We evaluated plant secondary compounds (here phenolics) since they have been shown to act as herbivore deterrents, repellents and/or attractants (Bernays and Chapman 1994). Phenolics such as phenolic acids and flavonoids seem to play important roles as induced and constitutive defences against herbivores (e.g., Ayres et al. 1997; Mumm and Hilker 2006). At the start of flowering in 2005 leaves were collected from 53 plants (27 females and 26 males), growing on island eight (Figure 1) in different successional stages for analyses of nitrogen and soluble phenolics. The plants from which the leaves were collected were chosen at random and categorised as attacked, unattacked or infected by *M. violaceum*.
For specific details of the material and methods, see the respective papers (Papers I-IV).

**Major results and discussion**

**Paper I**

In paper I we investigated whether there were any inter-sexual differences in attack rates of the larvae of two florivores *C. viscariella* and *D. criniventris* on male and female *S. dioica* plants on nine islands in the Skeppsvik archipelago across a period of seven consecutive years. Our results suggest an exception to what can been referred to as the ‘rule of male-biased herbivory’ (Cornelissen and Stiling 2005). We found significantly higher proportion of attack by the larvae of both florivores on *S. dioica* females compared to males (for 2000-06: 20% females and 18% males, Figure 3). When separate analyses of the attack frequencies in 2004-2006 were performed the same pattern emerged, females sustained higher proportion of attack of both *D. criniventris* (32.8% females and 30.7% males) and *C. viscariella* (4% females and 2% males) (Figure 3). This pattern was consistent over years and islands despite the strong spatial and temporal variation in flowering and attack frequencies we observed over the survey period (Figure 2, 3 and 4, in paper I). We complimented these data with analyses of plant nutrient status, secondary chemistry and morphological traits that might reflect sexual difference in host resource allocation to see if the observed difference in florivore attack was associated with some indicator of host quality. First, we found that, regardless of sex, plants with more floral stems had higher probability to get attacked (Figure 5, in paper I). Second, females had significantly more floral stems than males (2.22, and 2.08, respectively). Third, females had wider stems and stem leaves. However, we found no sexual dimorphism in nitrogen or total phenolics. These inter-sexual differences in stem number and the morphology of the stem might to some extent explain the higher attack rates on female plants. More stems may also ensure that the larval development is completed for *D. criniventris* larvae since they are able to move between stems. The sexual dimorphism in stem width and stem leaf width (see above), may suggest that females provide more of a better food resource and maybe also offer an increased shelter from predators and parasitoids for the larvae.
We also found a consistent female biased sex–ratio during most years and on all islands in our study area. This is not a common pattern in dioecious plants. In a study by Delph (1999) only 6 species out of 44 dioecious species showed female biased sex ratios. However, more striking is our findings that females are predominant in all life history stages, indicating that the herbivores in our study area are not the cause of the female biased sex ratio which has been invoked as an explanation of female biased sex ratio in other studies (Lovett Doust and Lovett Doust 1985; Wolfe 1997; Åhman 1997). Instead the female predominance could select herbivores to attack females more often since not only are females better as a food resource, with more stems, more food and better shelter, females are also more abundant in every life history stage. We thus concluded that the females seem to be the superior resource for the two herbivores in our system and the pattern observed in the survey supports this. For the two herbivores stem number seems to be the trait that influence the resource quality and since females have more and wider stems this indicates that females are the better resource.

**Figure 3.** Proportion of *Delia criniventris* and *Caryocolum viscariella* attacked floral female (F) and male (M) *Silene dioica* plants. White bars represent attack from both florivores pooled during 2000-2006; grey bars represent *D. criniventris* attack during 2004-2006 and dashed bars represent *C. viscariella* attack during 2004-2006. Data have been pooled for all islands.
This means that we need to focus on identifying the dimorphism responsible for the sex sustaining the greatest attack rates and avoid being blinded by the expectation of male herbivory.

**Paper II**

In paper II we investigated whether there were any inter-sexual differences in response to florivory between male and female *S. dioica* plants. We showed that the main effects of florivory by *C. viscariella* and *D. criniventris* on *S. dioica* are on the re-flowering capability the year following an attack. We found no effect on survival probability. Non-attacked females had an estimated mean re-flowering rate of 30.2%, and non-attacked males of 31%. Florivore attacked females had an estimated re-flowering rate of 46% and florivore attacked males of 38.4% (Figure 4). We found the survival probability to be uniformly high and there was no difference between males and females or any effect of florivore attack on survival probability (Figure 4). Our data also showed that attacked females with up to three shoots produced approximately half the number of capsules compared to non-attacked females (Figure 5). There was a positive effect on plant fitness in that attacked plants re-flowered to a higher degree the year following an attack compared to non-attacked plants. This means that attacked plants had yet another reproductive opportunity in the following year after an attack and therefore might not lose in life time fitness compared to non attacked plants. We also found a difference in degree of compensatory response between males and females i.e. females tended to re-flower more often than males and therefore showed a stronger compensatory response to florivory. This difference in the degree of re-flowering probability between attacked males and females might be a result of difference in timing of allocation of resources for reproduction (Delph 1999). In a sense females save more energy than males and are thus able to compensate more than males. Further, we also found a negative effect of florivory as females lost in capsule production and thus produced fewer seeds. Therefore attacked females might lose opportunities to spread their offspring in the year of attack however, the increase in re-flowering probability may give rise to more flowering events during their life time compared to non attacked females.
Figure 4. The estimated mean re-flowering probability (top) and the estimated mean survival probability (bottom) for females and males in the year after a flowering event. Grey bars denote non-attacked females and males and dashed bars denote males and females attacked by *D. criniventris* and *C. viscariella*. Data are pooled for all years.
Figure 5. Mean number of capsules produced by non-attacked (grey bars) and D. criniventris and C. viscariella attacked (dashed bars) females.

Paper III

The aim of paper III was to study changes in herbivory on Silene dioica across primary successional zones. Variations in nutrient, light and water availability between zones may have direct impacts on plant growth and consequently on plants size, nutrient quality and secondary chemistry (e.g., Herms and Mattson 1992; Bernays and Chapman 1994; Awmack and Leather 2002). Quality variation may thus affect the palatability of the plant for the herbivores. Host patch density has also been demonstrated to influence herbivore occurrence and it was hypothesized by Root (1973) that herbivores should accumulate in large dense and pure host patches. To evaluate difference in attack rates between plants growing in different successional zones, we studied the pattern of attack of D. criniventris and C. viscariella on S. dioica plants situated in young, intermediate and old successional zones on nine islands in a the archipelago over seven consecutive years. Our result shows that plants in young zones are significantly more attacked than plants in intermediate and old successional zones. 31% of the plants in young zone were attacked, whereas 27 % and 23% of the plants in intermediate and old zones were attacked, respectively (Figure 6). We also investigated the density of flowering plants per 5x5 m plot to asses if herbivores attack proportionally more plants in dense host patches. However,
intermediate zones had higher densities of floral plants compared to young zones while no difference was found between the young and old zones (Figure 6). This result indicates that the herbivore attack pattern is not correlated to host plant density per se in this system. We complimented these data with analyses of plant nutrient status, secondary chemistry and plant size (number of floral stems) in plants situated in the different successional zones.

Figure 6. The density (top) and the proportion of *D. criniventris* and *C. viscariella* attacked (bottom) floral individuals in the different successional zones. Data on all islands and years pooled.
We found that plants from young zones were larger, contained more nitrogen and had lower concentrations of phenolics compared to old zones. The relative importance of plant size, nitrogen content and secondary compounds cannot be evaluated from our data, although the ways in which these traits are associated with herbivore attack in our system are in the accordance with the plant vigor hypothesis (Price 1991; Price 1994). This hypothesis states that insects, for which their larval development is associated with processes of host growth, will be more abundant on plants/plant parts that are more vigorous, as they offer a resource of higher quality. To conclude, the herbivores in our system appear to attack plants in a quality depended way and not in a quantitative way. However there are other possible additional explanations for the pattern we see. For full discussion see paper III.

Paper IV

In paper IV we investigated the interactions between the anther smut fungus *Microbotryum violaceum* and *S. dioica*. We were particularly interested in whether the disease attacked one sex more than the other. We also investigated disease levels across a primary successional gradient and if there was a shift in intersexual difference in disease levels between the zones. To evaluate difference in disease frequencies between males and females we studied the interaction on nine islands the archipelago. The difference in disease frequencies between plants growing in young, intermediate and old successional zones were studied on three islands in the archipelago. Both studies were conducted over seven constitute years. We found significantly higher proportion of diseased females (6.1 %) compared to males (5.5 %) (Figure 7). This pattern is in accordance with most other field surveys of the *Microbotryum-Silene* association (Lee 1981; Alexander and Antonovics 1988; Shykoff et al. 1996; Kaltz and Shykoff 2001). It is also in agreement with the results in paper I, where the two herbivores, *C. visciariella* and *D. criniventris* also attacked females to a higher extent. However, in paper I the attack pattern was consistent over islands and years, while the disease frequencies varied among islands and in 2003 females had a lower disease frequency compared to males (see Figure 3 and 4 in paper IV). The different pattern in 2003 coincided with a drop in flowering frequency that followed a period of exceptional weather conditions, namely a prolonged drought.
the previous summer and autumn, 2002, followed by a cold winter with poor snow cover, 2002/2003 (http://www.tfe.umu.se/weather/1024/1050/10/sv-SE/history.htm 2008). The difference in disease frequencies among islands may be explained by disease levels. We found that disease was male biased on islands with the lowest disease levels, while on five of the six islands with higher disease frequencies the disease showed a female bias (see Figure 4 in Paper IV). Similar patterns, where the disease tend to shift from male- to female bias at higher disease frequencies, have also been reported in field surveys of *Silene latifolia* (Biere and Honders 1998; Kaltz and Shykoff 2001) If disease prevalence is low, infectious contacts are likely to be rare; under such situations a smaller floral display (characteristic for females) may keep spore deposition below critical levels (Roche et al. 1995; Giles et al. 2006) and thus males that are more attractive (have a larger floral display) will become similarly or even more often infected (Kaltz and Shykoff 2001). In contrast, when disease levels are high, contamination of both sexes with fungal spores may be so frequent that females, whose flowers remain attached to the plant for longer periods of time, will have higher infection rates (Shykoff et al. 1996; Kaltz and Shykoff 2001). One important mechanism for the switch from male bias to female bias in parallel to increasing disease frequency is thus that fungal spores have more time to initiate a successful infection on female flowers because fertilised flowers remain attached to the plant during the fruiting period, whereas male flowers are shed within a couple of days (Shykoff et al. 1996; Kaltz and Shykoff 2001).

Our result show that plants in the young zones are significantly less diseased compared to plants in intermediate and old successional zones. 8.3% of the plants in young zone were diseased, while 20.4 % and 13.6% of the plants in intermediate and old zones were diseased, respectively (Figure 8). This pattern of disease seems to correlate well with the density of flowering individuals (Figure 6). We also found that females were consistently (2-3%) more diseased than males in each of the three successional zones (see Figure 6 in paper IV). We did not find the expected shift in proportion disease between the sexes over the successional gradient.
Figure 7. The proportion *Microbotryum violaceum* diseased females and males. Data on all years and all islands pooled.

Figure 8. Proportion diseased individuals in the three successional zones. Data have been pooled for three islands and all years.
Pathogen-herbivore interaction

In our system the teliospores of the smut fungus *M. violaceum* are transmitted via pollinators to host plant flowers (Carlsson-Granér 1997). If the smut manages to infect and grow through the flower stalk and into the root system before the winter the plant will become systematically infected. Infected male and female plants produce flowers with anthers that are filled with fungal teliospores (Fischer and Holton 1957). When herbivores feed on floral stems the overall amount of stems is reduced and thus the chance of attracting the disease is reduced. In field experiment with *Silene latifolia*, plants that became infected with *M. violaceum* had larger numbers of flowers than individuals that remained healthy (Thrall and Jarosz 1994a) suggesting that plants with smaller floral display size, for instance due to loss of floral stems to herbivore damage will attract fewer pollinators and thus escape infection. Also the timing of herbivory is of importance. Early season herbivory might reduce the chance for successful transmission of spores and later herbivory might reduce the chance of establishing a systemic infection due to interruption of the infection process. Both types of herbivory will however reduce the probability of getting infected and spreading disease. To evaluate the interaction between *M. violaceum* and mountain hare (*Lepus timidus*) and roe-deer (*Capreolus capreolus*) we built enclosures to exclude the herbivores. We expected to see a higher increase of disease in enclosures since plants were protected from grazing of the floral stems and as a result slower increase in disease frequencies outside the enclosures than inside the enclosures. We studied this interaction on four islands (islands 4, 5, 6 and 7; Figure 2) and excluded islands with only a few diseased plants in the enclosures. We found a weak tendency that disease frequencies within enclosures either increased more or decreased less compared with outside the enclosures (Figure 9). This suggests that excluding grazing of floral stems will increase the disease frequencies; however, a longer time period seems to be needed until this experiment can be evaluated.
In paper IV we also, briefly, discussed the possibility that there is a negative interaction between *M. violaceum* and the two floral stem eaters *C. viscariella* and *D. criniventris* in our study system. Our data suggest some competitive interaction for the shared resource as the two herbivores attack healthy plants more often than diseased plants, 34 and 18 %, respectively \((\chi^2_1=27.6)\). Also it is worth observing that the two insect herbivores show high attack rates in the younger populations (Paper I) and in young successional zones (Paper III) where disease frequencies generally are low. We conclude that an increased ambition aiming to understand species interactions embedded in more natural and complex settings will be of great importance to understand the nature of changing selection pressures operating on natural enemy-host plant associations.

**Conclusions**

Long time studies like ours are important in that they pick up small but important differences in the interactions between host plants, herbivores and pathogen that might be lost in short time studies. These interactions might be important when assessing the implications of individual plants fitness and more important the influence of herbivores and pathogens on host plant populations fitness.
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