TROPHIC, INDIRECT, AND EVOLUTIONARY INTERACTIONS IN A PLANT–HERBIVORE–PARASITOID SYSTEM

Johan A. Stenberg

2008

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 erhållande av filosofie doktorsexamen i ekologi kommer att offentligen försvaras fredagen den 8 februari 2008, kl. 10.00 i Lilla hörsalen, KBC.

Examinator: Professor Lars Ericson, Umeå universitet

Opponent: Dr. John D. Parker, Smithsonian Institution, Edgewater, MD, USA

ISBN: 978-91-7264-469-4
© JOHAN A. STENBERG 2008
Printed by VMC, KBC, Umeå University, Umeå 2008
The aim of this thesis project was to elucidate patterns and processes associated with the biotic interactions in a natural plant–herbivore–parasitoid food web characterized by spatial and temporal heterogeneity with regard to species composition.

The system examined is based on island populations of the perennial herb Meadowsweet (*Filipendula ulmaria*, Rosaceae), located in the Skeppsvik Archipelago. The area is subject to isostatic rebound, amounting to 0.85 cm per year; this makes it possible to calculate the age of the rising islands. Meadowsweet colonizes new islands when they are about 100 years old.

Meadowsweet is consumed by two major herbivores in the study area: *Galerucella tenella* and *Altica engstroemi* (Coleoptera: Chrysomelidae). Both herbivores overwinter in the topsoil and successful colonization occurs when the islands reach a height that prevents the beetles from being removed or killed as a result of wave wash during the winter. I found that both herbivores significantly reduced individual plant fitness and population growth rate. A “cafeteria experiment” with *Galerucella* showed that this beetle discriminated between plants from different islands, avoiding plants from old islands which contained high concentrations of putative defence compounds, while readily accepting plants from younger islands which contained lower concentrations of these chemicals. Further, the plant species exhibited a trade-off between growth and production of the putative defence compounds. Taken together, these results were interpreted as providing evidence of herbivore-driven evolution of resistance in Meadowsweet. Further, laboratory studies suggested that *Galerucella* gradually includes a less preferred host plant (*Rubus arcticus*, Rosaceae) in its diet as Meadowsweet resistance increases. This implies that *Galerucella* drives its own host-breadth enlargement by selectively inducing a ‘rent rise’ in the original host, Meadowsweet.

In a number of field studies I showed that the oligophagous parasitoid *Asecodes mento* (Hymenoptera: Eulophidae) has a strong positive effect on Meadowsweet seed set by removing large numbers of *G. tenella* larvae. This top-down effect is, however, altered by the presence of a close relative of *G. tenella*, namely *G. calmariensis*, which is monophagous on Purple loosestrife (*Lythrum salicaria*, Lythraceae). *G. tenella* experiences associational susceptibility when coexisting with *G. calmariensis* since the latter supports a higher and more fit pool of shared parasitoids and because Meadowsweet attracts a higher proportion of the shared parasitoid females than Purple loosestrife. This implies that *G. tenella* densities are very low in coexisting populations and that Meadowsweet experiences associational resistance and produces more seeds when co-occurring with Purple loosestrife. Thus, selection for increased resistance in Meadowsweet is likely to be relaxed in populations mixed with Purple loosestrife.

I conclude that the evolution of plant resistance is likely to depend on the length of time and intensity of selection. When Meadowsweet colonizes new islands it experiences a period of enemy-free space; followed by a midlife and ageing with selection by herbivores. The intensity of this selection does, however, depend on the presence of additional plant and herbivore species.

**KEY WORDS:** Plant resistance, coevolution, herbivory, Skeppsvik, food web, parasitism, maternal effect, natural selection, *Filipendula ulmaria*, Chrysomelidae.

**LANGUAGE:** English **ISBN:** 978-91-7264-469-4 **NUMBER OF PAGES:** 16 + 5 papers

**SIGNATURE:**

**DATE:** 2008-01-07
TROPHIC, INDIRECT, AND EVOLUTIONARY INTERACTIONS IN A PLANT-HERBIVORE-PARASITOID SYSTEM

Johan A. Stenberg

2008

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

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

I  Stenberg JA, Lind M & Ericson L (submitted manuscript) Insect herbivory controlled plant fitness and population growth in a 16-year study of Meadowsweet populations.


Papers II–V are reproduced by kind permission of the publishers.
INTRODUCTION

From the beginning of the 20th century to the present day, the extent to which insect herbivores affect the individual fitness, demography and evolution of plant hosts has been hotly debated (Ehrlich and Raven 1964, Janzen 1980, Jermy 1993, Bigger and Marvier 1998, Zangerl and Berenbaum 2005, Maron and Crone 2006). Most research has focused on interactions between cultivated agricrops and their associated insect pests, which can reduce crop yields and lead to severe economic losses and problems with feeding the increasing human population. Such agriecosystems are usually ecologically simple, consisting of large monocultures of plants with only a few associated insect species. Moreover, natural resistance to herbivores is likely to be compromised in these crops as a result of intense human-induced selection during cultivation (Welter and Steggall 1993, Rosenthal and Welter 1995, Rosenthal and Dirzo 1997). In addition, the evolution of the insect pests involved is likely to respond more to commonly used industrial insecticides than to the weakened (compromised) intrinsic resistance of the plants involved (Mallet 1989), making most aspects of ecology and evolution in these systems extremely unnatural, and of little relevance to more fundamental studies of plant–insect relationships.

Less effort has been exerted to increase our understanding of plant–insect interactions in natural food webs in the overwhelming complexity of natural environments. In part, this has clearly been due to a lack of economic incentives for studying natural systems, but also because the intrinsic complexity is difficult to deal with. In comparison to agriecosystems, natural systems usually include co-occurring plant species, and a much wider diversity of herbivores and carnivores. This makes the number of direct and indirect interactions potentially very large and, it has been argued, the effect of individual interactions, therefore, low. In particular, it has been argued that individual herbivore species are of little importance to their plant hosts (Janzen 1980, Jermy 1993), while plants are of immense importance to their insect herbivore tenants (Pappers et al. 2002, Feder et al. 2003, Weingartner et al. 2006, Stenberg and Axelsson 2008), making individual insect–plant interactions highly asymmetric.

However, although the number of insect species interacting with any given plant may be very large on the regional scale, researchers have increasingly emphasised that plants, as well as their associated herbivores and carnivores, are usually heterogeneously distributed in time and space (e.g. Thompson 1994, Hanski 1999, Thompson 2005). It follows that species compositions may vary greatly between patches and years and, hence, that individual players may be important selectors in certain patches and unimportant in others. Similarly, although herbivory may be negligible in some, or most, years, crucial selection pressures may be exerted during years of herbivore outbreaks. Hence, biotic selection will vary in time and space, suggesting that it is of the utmost importance to consider the spatial and temporal scale at which to undertake investigations when studying plant–insect interactions. If restricting the investigation to a particular year or patch, one may seriously misunderstand, or underestimate the importance of insect herbivores in shaping the demography and evolution of plant populations.
This thesis project was largely inspired by John N. Thompson, who suggested that species traits may be locally shaped and reshaped by biotic selection organised in a “geographic mosaic of coevolution” (Thompson 1994, 1999, 2005). In this scenario local selection in combination with migration, genetic drift, and founder effects may create a more or less random mosaic of traits, interactions and lack thereof.

Building upon Thompson's ideas, a starting point for this thesis was that although the distribution patterns of species traits and interactions form a geographic mosaic, this does not mean that the distribution is necessarily random. In fact, if it is possible to derive information about population founders and the subsequent history of species composition in local populations, and, if the factors influencing the strength of biotic selection can be elucidated, then it should be possible to predict the evolutionary products of local biotic interactions. With this in mind, I decided to examine the patterns and mechanisms associated with the interaction between the perennial host plant, Meadowsweet (*Filipendula ulmaria*, Rosaceae) and its major herbivore (*Galerucella tenella*, Coleoptera: Chrysomelidae) in an archipelago undergoing land uplift. This area contains about 100 islands (representing the spatial dimension) that are characterised by a predictable colonisation process (the time dimension). This system (see Study system) is unique and perfect for studying aspects of coevolution because it is possible to estimate the aforementioned factors, which are believed to determine the evolutionary products of local biotic interactions.

I began by investigating the effects of natural leaf-beetle herbivory on the fitness and demography of Meadowsweet. I then studied direct (chemical) and indirect (parasitoid attraction) defences and examined how these were distributed across space and time in the archipelago. The effect of plant resistance on *Galerucella* performance and preference was also tested, including whether Meadowsweet plant resistance affected the selections made by *Galerucella* and if so, the level of impact. Since my results indicated that herbivores escape from resistant Meadowsweet individuals, I continued to investigate the possibilities for host-breadth enlargement of *Galerucella* on islands with highly resistant Meadowsweet populations.

In the next phase, I investigated the factors influencing the density of *Galerucella* in Meadowsweet populations. It soon became clear that a single parasitoid species, *Asecodes mento* (Hymenoptera: Eulophidae) was extremely important as a top-down regulator. However, since this parasitoid is oligophagous and parasitizes a close relative to *G. tenella*, namely *G. calmariensis* which in turn is monophagous on Purple loosestrife (*Lythrum salicaria*, Lythraceae), an investigation was undertaken in order to elucidate the possible indirect interactions between the two parallel food webs and their impact on Meadowsweet plants.

**STUDY SYSTEM**

The Skeppsvik Archipelago is located on the western side of the Gulf of Bothnia, Sweden, approximately 20 km east of Umeå (62°42’N, 20°25’E). The area is subject to land uplift (0.85 cm/year) and comprises about 100 islands in different stages of succession, ranging from young incipient islands to old forest-covered islands more than 1,000 years old (Ericson 1981). Due to the uplift, new islands are continuously formed and colonized by vascular plants (Valovirta 1937, Carlsson et al. 1990), which in turn are colonized by various natural enemies (Carlsson et al. 1990). In
general, host plant colonization is highly predictable mirroring the height (age) of an island and its location within the archipelago (Carlsson et al. 1990), while colonization and persistence of host-specific natural enemies correlates with host population size (Carlsson et al. 1990, Burdon et al. 1995).

Meadowsweet (*Filipendula ulmaria*, Rosaceae) is a perennial herb that dominates the upper part of the open shores. Plants reproduce by seeds that germinate in early summer and by slow lateral spread of the rhizomatous rootstock.

*Altica engstroemi* (Coleoptera: Chrysomelidae) is monophagous on Meadowsweet. It is usually confined to the inner part of the archipelago, and is usually found at high densities and is only occasionally observed elsewhere in the archipelago. The adults emerge in late May and feed on the emerging new leaves. The larvae emerge in late June and feed on the leaves, which become skeletonised. Several colour morphs of *A. engstroemi* exist in the archipelago, and they seem to interbreed.

In the outer and middle parts of the archipelago, *Galerucella tenella* is confined to Meadowsweet, while in the innermost part of the archipelago it also attacks two other species of Rosaceae: *Rubus arcticus* and *Potentilla palustris*. Feeding experiments showed that Meadowsweet is strongly preferred. *G. tenella* is regularly found on most of the larger islands, but shows marked asynchronous density variations between years. The lifecycle of *G. tenella* is similar to that of *A. engstroemi*. However, in contrast to *A. engstroemi*, it is frequently found in inflorescences during the flowering period, where it feeds on flowers and seed embryos. *G. tenella* larvae are heavily attacked by the parasitoid wasp *Asecodes mento* Walker (Hymenoptera: Eulophidae) which is likely to be one important explanation for the population asynchrony.

*A. mento* (Hymenoptera: Eulophidae) is a koinobiont larval endoparasitoid that is known to attack young larval stages of all species of the genus *Galerucella* as well as species in related chrysomelid genera. In the archipelago studied, its only hosts are *G. tenella* and *G. calmariensis* L., the latter being monophagous on Purple loosestrife (*Lythrum salicaria*, Lythraceae). *A. mento* overwinters as pupae in mummified host larvae in the ground. Most adults emerge in July when they parasitize *Galerucella* larvae. A few adults emerge from the mummified host larvae the same summer, comprising a small second generation, but most remain as pupae in their hosts, entering diapause (Stenberg & Hambäck, submitted manuscript).
OBJECTIVES OF THE THESIS

The objective of the research was to answer the following questions:

- Does chrysomelid herbivory affect the individual fitness and population growth rate of Meadowsweet? (papers I and V)
- Do chrysomelids selectively induce an evolutionary response to herbivory in Meadowsweet? (paper II)
- Is Galerucella tenella locally adapted to its host plant populations? (paper II)
- Can herbivores drive their own host-breadth enlargement by selectively inducing a “rent rise” in their original host plant? (paper III)
- Do parasitoids control the amount of herbivore damage on Meadowsweet in a top-down fashion? (papers IV and V)
- Do co-occurring plants and herbivores exert associational effects on the plant–herbivore–parasitoid system? (papers IV and V)

MATERIALS AND METHODS

Understanding a plant–herbivore–parasitoid system requires methods to isolate insect behaviour as well as plant chemistry, demography and much more within the system. This requires a plethora of approaches. In the research for this thesis I used a combination of experiments in the field and in the laboratory, as well as observational studies to examine the questions listed under “Objectives of the thesis”.

The observational studies included: (i) scoring population sizes and herbivore damage in a number of Meadowsweet populations over time; (ii) collecting and rearing G. tenella and G. calmariensis larvae to study the patterns and phenology of parasitism throughout the archipelago; (iii) measuring the amount of G. tenella damage and seed production for Meadowsweet on islands the year after parasitism data were obtained; and (iv) determining the chemical composition of Meadowsweet leaves from different islands.

The experimental studies included: (i) removing herbivores from naturally growing Meadowsweet plants; (ii) a number of “cafeteria experiments” with the chrysomelids; (iii) performance studies of the chrysomelid larvae and adults; and (iv) behavioural assays of the parasitoids.

For specific details of the materials and methods, see the attached chapters.
MAJOR RESULTS AND DISCUSSION

My results clearly show that insect herbivores may be very important for plant fitness in natural systems. High levels of chrysomelid herbivory were associated with a significant reduction in seed production and even mortality of individual Meadowsweet plants (paper I). I also found evidence for lower performance of the offspring (seeds) of damaged mother plants. This reduced fitness of herbivore-damaged plants results in reduced population growth rate of Meadowsweet at both Altica engstroemi and Galerucella tenella sites in Skeppsvik. Paper I provides a rare contribution to this research area, since long term studies comparing the effects of insect folivores and inflorescence feeders on natural perennial populations are almost unknown in the ecological literature. A surplus of safe sites, along with a diluted seed bank, probably contributes to the major effect of herbivory on the plant population growth rate in this system (Maron and Crone 2006).

The pronounced fitness effect of chrysomelid herbivory is also likely to affect the structure of the plant populations. In paper II I showed that individual Meadowsweet plants varied with respect to leaf chemistry and that G. tenella moved away from plants that contained high concentrations of resistance compounds which slowed down larval development. From these results I predicted that the evolution of plant populations would be affected by selective chrysomelids which reduce individual plant fitness. Accordingly, plant resistance was positively correlated with island age, corresponding to the time of exposure to herbivore selection. I feel, therefore, that it is reasonable to suggest that the chrysomelids selectively induce a higher mean resistance in colonized Meadowsweet populations over time.

While paper II suggested that the herbivores have an evolutionary effect on Meadowsweet, it does not provide any support for coevolution (Janzen 1980), i.e. the chrysomelids did not seem to be locally adapted to individual plant populations. In general, researchers have suggested that the evolution of insect herbivores should be strongly affected by their host plants, while the evolution of host plants should be more or less independent of insect herbivores. The results from paper II seem to contradict these suggestions. However, one must realize that the spatial scale is a crucial factor when examining local adaptation, and it is certainly possible that the G. tenella in Skeppsvik comprise one single outbreeding population and that adaptation to host plants should be studied at larger spatial scales. This is supported by Stenberg & Axelsson (2008) who suggested that G. tenella has evolved different host races on Meadowsweet in Skeppsvik and Strawberry in Finland (Fig. 1). It may, therefore, be inaccurate to conclude that Meadowsweet and G. tenella are not coevolved, although the interaction is not likely to be fully symmetrical.

In addition to the present Meadowsweet–chrysomelid system, at least one additional well described system exists were an insect herbivore has driven the contemporary evolution of plant resistance in natural populations (Zangerl & Berenbaum, 2005). In the present Meadowsweet–chrysomelid system, however, I went on to predict the consequences of herbivore-driven evolution of plant resistance. Rather than leading to an arms race, I suggested in paper III, that in Skeppsvik G. tenella escapes from
the increasingly resistant Meadowsweet plants and increases its use of alternative host plants. However, once again one has to keep the patchy species distribution in mind. In the outer part of the archipelago, where Meadowsweet resistance is generally low, no alternative host plant to *G. tenella* exists. Furthermore, on these islands *G. tenella* generally have no need for alternative hosts since most Meadowsweet individuals are susceptible and of high quality for use by the herbivore. However, alternative host plants frequently occur in the inner part of the archipelago where Meadowsweet resistance is generally higher, and consequently *G. tenella* adjusts its host use accordingly. These patterns largely support the geographic mosaic of coevolution theory (Thompson 1994, 1999, 2005), but allow us to be optimistic regarding the possibility of predicting specific patterns in the mosaic when background information is available.

So far we have learnt that *G. tenella* is an important factor affecting Meadowsweet. However, natural systems are generally more complex than just a single host plant and one or two herbivores. In order to grasp the true field situation, carnivores and
co-occurring plants and herbivores also have to be considered (Agrawal et al. 2007). As we shall see, parallel food webs are of the utmost importance for herbivore pressure on Meadowsweet. The parasitoid Asecodes mento seems to be a significant enemy of G. tenella in most parts of northern Europe. In papers IV and V I showed that this species parasitizes up to 100% of G. tenella larvae in Skeppsvik and that it has a delayed bodyguard effect on Meadowsweet through trophic cascading. If the bodyguard service delivered by a local parasitoid population is looked upon as a resource, then we can envision that this resource can be consumed and competed for by co-occurring plants. In the case of A. mento, it is oligophagous on Galerucella species and in Skeppsvik it has only two hosts, G. tenella and G. calmariensis; the latter being monophagous on Purple loosestrife (Lythrum salicaria, Lythraceae). In paper V I showed that the parasitoid makes use of odours released from the respective plants to orient itself to its prey, and that Meadowsweet odours were particularly enticing, thus attracting the larger proportion of the shared parasitoid pool. As species distributions are heterogeneous in Skeppsvik, islands that host either mixed or monospecific plant populations can be found. In accordance with the results pertaining to parasitoid behaviour, I found that parasitism of G. tenella was higher in mixed populations where Meadowsweet enjoys associational resistance due to the inflow of parasitoids from G. calmariensis hosts on Purple loosestrife. The associational resistance, however, is not only an effect of parasitoid behaviour. Stenberg & Hambäck (submitted manuscript) also showed that parasitoid resource competition within hosts is of the utmost importance for the size (fitness) of emerging parasitoids. G. calmariensis is the larger of the two hosts and, thus, parasitoids experience lower resource competition in this host, leading to larger (fitter) parasitoids flowing from G. calmariensis to G. tenella. Larger parasitoid females are, in turn, likely to live longer, produce more eggs, and be more efficient than small females. Hence, the asymmetric apparent competition between G. tenella and G. calmariensis is likely to be the result of a combination of adult parasitoid behaviour and parasitoid resource competition within individual host mummies. Ultimately this apparent competition leads to a lower herbivore pressure on Meadowsweet individuals that occur with Purple loosestrife. As a matter of fact, G. tenella almost never builds up large numbers in mixed populations, while monospecific populations fluctuate quite asynchronically. The degree to which the relaxation of herbivory (due to the presence of Purple loosestrife) affects the evolution of resistance in Meadowsweet remains to be studied, but it is likely to add more complexity to the geographic mosaic of coevolution.

CONCLUSIONS

The most striking finding of my studies is that insect–plant interaction patterns can vary over very small spatial and temporal scales. Simply speaking, Meadowsweet populations enjoy beetle-free space on new islands for less than a century, during which selection favours susceptible plant individuals until the island reaches a height that allows the beetles to establish, after which selection favours resistant plant individuals. However, if the island is colonised by Purple loosestrife as well, then selection pressures from G. tenella will remain negligible. This story is acted out on a stage of just a few kilometres over a few hundred years. My results do not suggest
that the presumed evolutionary products of this scenario are counteracted by migration, genetic drift, or other balancing forces.

ACKNOWLEDGEMENTS

I am most grateful to my supervisor Prof. Lars Ericson for his valuable cooperation, and supervision. I would also like to thank the coauthors of the chapters in this thesis, who, in addition to Lars Ericson, include Peter A. Hambäck, Juha Heijari, Jarno K. Holopainen, Martin I. Lind, and Johanna Witzell; they provided stimulating collaboration on various parts of this PhD project. I am also thankful for the collaboration of E. Petter Axelson and Ian T. Baldwin during this period, which resulted in papers that have been published elsewhere. Last, but not least, I want to thank Kjell Leonardsson, Jonas Dahlgren, Johan Olofsson, Hans Gardfjell, and the R study group who provided statistical support and Sees-editing Ltd who corrected all my English texts. Financial support was provided by the Swedish Research Council VR (to Lars Ericson), the European Science Foundation, the German Academic Exchange Service (DAAD), the Swedish Institute, Gunnar and Ruth Björkman Foundation, Helge Ax:son Johnson Foundation, J C Kempe Academic Foundation, Gustaf and Hanna Winblad Memorial Foundation, and NorFA.

REFERENCES


Stenberg JA & Hambäck PA (submitted manuscript) Host species critical for offspring fitness and sex ratio for an oligophagous parasitoid: implications for host coexistence.


