

# Interactions and Coevolution in Tritrophic systems

Lisa Fors



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Cover: Members of a tritrophic system. Left: *Lythrum salicaria* Photo: Ellen Schagerström,  
Top right: *Galerucella californiensis* Photo: Robert Markus, Bottom right: *Asecodes luscens*  
Picture by *UppsalaAnimation*.

## **Summary**

Ecological systems are usually complex, with a number of interacting species. These species interactions are commonly divided into two major groups: mutualistic and antagonistic. If the interactions are mutualistic, they are beneficial for all species involved, as in specialized relationships between certain plants and their pollinators. Antagonistic interactions, on the other hand, can be either competitive or trophic. Trophically interacting species are for example plants and their associated herbivores, predators and their prey or parasites and their hosts. In many of these interactions, some species are depending on others in order to survive. If one species changes, other species associated to it may have to adapt to the changes. This may lead to a process of reciprocal evolution between the interacting species, called coevolution. In this paper I start with a brief background on coevolution and local adaptation, and then describe some interactions in tritrophic systems. The tritrophic systems I focus on consist of plants, herbivore insects and parasitoids. I discuss some processes and mechanisms in these systems, such as host search, plant defense and the immune response in insects. In the end of the paper, a short description of my PhD-project is included.

## **Sammanfattning**

Ekologiska system består vanligtvis av ett stort antal arter som interagerar med varandra på olika sätt. Ofta beskrivs dessa interaktioner som antingen mutualistiska eller antagonistiska. I mutualistiska samspel drar alla inblandade arter nytta av interaktionen, vilket till exempel är fallet hos specialiserade pollinatörer knutna till en viss växt. Antagonistiska interaktioner kan istället förekomma mellan konkurrerande arter eller i system med olika trofiska nivåer. Trofiska samspel förekommer till exempel mellan växter och herbivorer, predatorer och bytesdjur eller parasiter och värddjur. I många av dessa interaktioner är vissa arter beroende av andra för att kunna överleva. Om en art förändras kan andra arter kopplade till denna art behöva anpassa sig till förändringarna. Det kan leda till att utvecklingen hos interagerande arter följs åt i en slags växelverkande process, så kallad samevolution. Denna uppsats inleds med en beskrivning av samevolution och lokal anpassning och fortsätter sedan med exempel på olika interaktioner i tritrofiska system. De tritrofiska system jag i första hand fokuserar på består av växter, herbivora insekter och parasitoider. Jag diskuterar några av de processer och mekanismer som förekommer i dessa system, såsom sökandet efter värdväxter, växtförsvar och immunförsvaret hos insekter. Uppsatsen avslutas med en kort beskrivning av mitt doktorandprojekt.

## Introduction

In ecology, the study of interacting species is fundamental. If one species is dependent on another for survival, it naturally has to co-occur and interact with this species, as seen for example in predators dependent upon their prey or parasites depending on a specific host. Usually, ecological systems have several trophic levels with more than just two species interacting, either in a mutualistic or an antagonistic way. If one species changes, other species depending on it consequently need to adapt to these changes, which can lead to coevolution.

## Coevolution

In biology, the term evolution can be defined as the change over time, where new types of living organisms are developed from pre-existing types, as a result of the accumulation of genetic differences. Such differences can be driven by for example geographical barriers and reproductive isolation (Slatkin 1987). Much of evolution is coevolution between species (Thompson 1994). This is a process of reciprocal evolution between interacting species, driven by natural selection.

If the process of coevolution is idealized, it could be described as one population of one species coevolving with one population of another species in a single, more or less stable, environment. The coevolution between the two species will then lead to either local extinction of one species or a state of equilibrium. In reality, however, coevolution is much more complex, often involving several populations spread across shifting environments. In *classical coevolution* there is a reciprocal evolutionary change in a two-species interaction, whereas *diffuse coevolution* is evolution in the context of a whole community and not just species pairs (Thompson 1989). The *geographical mosaic theory of coevolution* also takes into account the spatial variation that occurs within populations, which means that there is a geographical pattern of coevolution shifting constantly between two or more species (Schoonhoven et al. 2005). This form of coevolution can be seen as a contribution to the speciation process and the differentiation of phylogenetic lineages (Thompson 2005).

Coevolution can be divided into mutualistic and antagonistic processes, reflecting how the involved species interact with each other. In mutualistic processes, all species involved

benefit, as in specialised plant-pollinator interactions. Antagonistic interactions can be either competitive; seen for example in plants trying to cope with locally competing species, or trophic; including plant-herbivore, plant-pathogen and predator-prey interactions (Kniskern & Rausher 2001).

A prerequisite for coevolution is local adaptation. Recently there has been an increasing interest for local adaptation driven by interactions between hosts and parasites (including small herbivores, parasitoids, pathogens and ectoparasites). Other species associated to the host-parasite system may also be affected by these interactions. There are several reasons why local adaptation may be particularly likely to occur in host-parasite systems. For example, selection imposed on parasites by host defences is strong, and parasites often impose strong selection on their hosts. Thus, host-parasite systems are well suited for studying local adaptation (Kaltz & Shykoff 1998).

### **Local adaptation**

Adaptation is an evolutionary process facilitating the survival of an organism in a specific environment (Williams 1966). Local adaptation can be defined as a genetic change in a population, due to geographically localized selection pressure. This occurs when there is a variation in selective pressures across the landscape. The selective pressures are determined by different factors, both biotic and abiotic. Examples of biotic factors are competing species and natural enemies. Abiotic factors can be soil type, light availability, differences in temperature and elevation etc. Other possible factors influencing local adaptation are distance among populations (spatial scales) and migration rates. Hence, gene flow can play an important role in the process of local adaptation, as it may prevent populations of the same species to evolve independently (Kawecki & Ebert 2004; Hereford 2009).

How well an organism is adapted is usually measured by its fitness. The individuals with highest fitness in a population are by definition those that leave the highest number of descendants. Local adaptation can result in a higher fitness in local individuals at their home site, compared to the fitness of nonlocal individuals. The fitness depends on the individuals' ability to cope with local conditions, both biotic and abiotic (Dickie & Moyersoen 2008). In locally adapted individuals, each phenotype corresponds to one specific environment. This differs from phenotypic plasticity, where a certain genotype has the ability to express different

phenotypes across different environments. Phenotypic plasticity is favoured by variability in the environment as well as by gene flow, whereas local adaptation is limited by gene flow (Ortegón-Campos et al. 2009), as migration between populations works as a homogenizing factor.

In plants, local adaptation also affects other species associated with them. Many insects have specific host plants, which are used for both feeding and oviposition. The quality of host plants is highly important to the fecundity of the herbivorous insects connected to them, both on individual and population level (Awmack & Leather 2002). Host plant quality is used to describe plant components affecting herbivorous insects, positively or negatively. Such components could be the levels of carbon, nitrogen and defensive compounds in the plant. Due to external environmental factors, such as changes between seasons or environmental stress, the quality of plants varies considerably. Additionally, the herbivorous insects themselves often change the quality of their host plants. This can have effects on both inter- and intraspecific interactions. Also higher trophic levels, such as predators and parasitoids, can be affected by host plant quality (Stoepler et al. 2011).

Populations of plants and insects can form local subpopulations with local adaptations. This process results in gene complexes that are locally adaptive and tightly linked. Consider for example a population of herbivorous insects that uses a certain host-plant. If the herbivores are given different opportunities in patches where their host plant shows different genotypes, subpopulations of the herbivore may be formed that are also genetically different from each other. Certain genotypes of the herbivore, better adapted to the local host-plant genotypes, will then be favoured by local selection. Genes that result in adaptation may be linked to each other and lead to co-adapted gene complexes. If individuals from different subpopulations hybridize, these gene complexes could break down, leading to outbreeding depression. This means that local mating is favoured, as hybrids usually have lower fitness (Schoonhoven et al. 2005).

## **Tritrophic systems**

Ecosystems consist of species at multiple trophic levels, e.g. levels in a food chain. The transfer of energy from one part to another in an ecosystem is referred to as trophic dynamics. In a trophic cascade, an organism at one level of the food chain can affect the occurrence of organisms at other levels (Poelman et al. 2008).

Interactions between plants and insects are common in ecosystems. Usually there are more than just two species involved in such interactions. One insect species may interact with several different plant species, or there might be several insect species connected to the same plant. A tritrophic system consists of three interacting levels, for example a herbivore insect feeding on a host plant and a predator or parasitoid attacking the herbivore. In such a system, several processes and mechanisms are of importance. To start with, the herbivore insect is interested in finding a host plant that can provide good nutrition and that is suitable for oviposition. At the same time, it is crucial for the herbivore to avoid attacks from natural enemies such as predators, parasites and parasitoids. For the plant it is naturally important to avoid the herbivore.

## **Parasites and parasitoids**

The most common life form on earth is constituted by parasites. The definition of a parasite is an organism that uses another living organism of a different species as food resource, either for one part of the parasite's life or during its whole lifetime, to the detriment of the other species. The parasite usually lives on or in the body or cells of its host organism, which in most cases is harmful to some extent to the host (Fig. 1). A parasitoid on the other hand, is an organism whose parasitic action always kills the host organism. Examples of parasitoids are insects that are free living as adults, but lay their eggs inside other insect larvae (Fig. 2). The parasitoid larvae develop inside the host larva, consuming its tissues and ultimately kill it. As parasites and parasitoids successfully reduce the fitness of infected hosts, there should be a selection for traits that could protect species against parasitism (Baucom & Roode 2011). In an interaction between a host and a parasitoid, the resistance of the host is defined as the probability that the host kills the parasitoid egg or larvae. Accordingly, the virulence of the parasitoid is the probability that it overcomes host defences (Green et al. 2000).





**Figure 1.** The apple maggot (*Rhagoletis pomonella*), is a pest of several fruits, mainly apples. Photo: Joseph Berger



**Figure 2.** The parasitoid *Peristenus digoneutis*, about to lay an egg in a plant bug nymph. Photo: public domain

### Host search

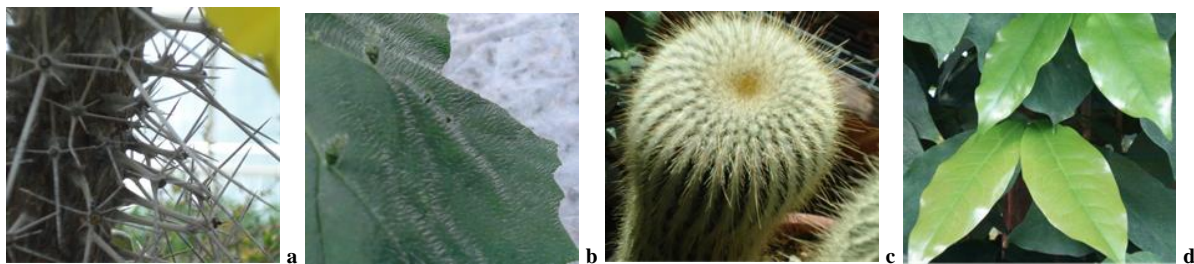
There is a distinction between host finding and host recognition. The former refers to the orientation of the insect and is affected by the distribution of the host, while the latter refers to the insect's decision to use a certain host for feeding and/or oviposition (Visser 1988). In many herbivorous insect species the larvae are incapable of moving to another host plant, at least as newly hatched, and have to feed on the plant where they were laid. The host selection by adult females is clearly of high importance for individual fitness, and many insects are highly discriminating when choosing plants for food and oviposition sites (Mackay 1985).

To locate a host plant, insects can use different search ways; visual, olfactory and gustatory or contact. Naturally, the importance of different types of stimuli varies between species and may also change with distance to the host plant. The most important stimuli for many insects are olfactory and visual cues, often used in a combination. Visual characteristics of plants that may influence host selection are shape, size and spectral quality. In contrast to olfactory cues, visual cues are not likely to be affected by abiotic factors such as wind and temperature, and should therefore be quite stable (Schoonhoven et al. 2005). However, if the vegetation is dense and complex, visual cues may not be very accurate. Olfactory signals are airborne chemical compounds, for example green leaf volatiles produced by plants. The dispersal of such signals is clearly affected by abiotic factors, primarily wind velocity. Plant odors are always complex blends, as numerous volatile compounds often leave the plant surface at the same time. Herbivorous insects have evolved a system for olfactory reception which enables them to distinguish some of the plant volatile in such a blend and translate them into a chemical message (Visser 1986).

When herbivorous insects feed on their host plant, the plant is stressed. This often leads to raised levels of some of the green leaf volatiles emitted, or production and release of new compounds. Thus, insects already feeding on a plant may attract even more conspecifics (or possibly other species) to the same plant, leading to herbivore aggregation. In addition to plant odors, volatile compounds are also produced by the insects themselves. Insects use odors not only for host search, but also as signals for social and sexual behaviour (Morris et al. 1996).

### Plant defense

In order to avoid or get rid of herbivores, plants can use different types of defense, both direct and indirect. The defense strategies can be morphological features, such as thorns, spines or trichomes (Fig. 3a-c), smooth and slippery leaves (Fig. 3d), tissue toughness, wax cover and resin secretion (Howe & Schaller 2008).



**Figure 3a-d.** Examples of defense strategies in plants. Photos: Lisa Fors

The plant can also use secondary metabolites as a chemical defense against herbivory, including terpenoids, alkaloids, phenolics, cyanogenic glucosides and glucosinolates. The role of defense chemicals in the plant is to impair the performance of the herbivore, either by reducing the plant's nutritional value or by acting as toxins or feeding deterrents. Some defense traits are expressed constitutively whereas others are inducible triggered for example by an attack of herbivorous insects (Howe & Schaller 2008). Induced defense includes production of organic volatiles and changes in phytochemistry or plant morphology.

Antagonistic plant-insect interactions of this type lead to selection on plant defense traits. This has mainly been studied for interactions in systems consisting of one plant and one herbivore. However, other members in a plant-based community, such as predators or parasitoids attacking the herbivore, are also affected by these traits. For example, natural enemies of the herbivore such as parasitoids might be attracted by green leaf volatiles omitted by the plant in response to herbivore damage. The parasitoids may use these chemical signals to locate

possible insect hosts for their offspring; the release of the volatile compounds can then function as an indirect defense for the plant. Chemical defenses in a host plant could also affect the herbivores' immune response against parasitoids. It has been shown that herbivorous larvae feeding on plants with low concentrations of chemical defenses have a better ability to withstand parasitoid attacks than those feeding on plants with high concentrations (Poelman et al. 2008).

## **Insect immunology**

### *Ecological factors influencing the immune system in insects*

The immune system of an organism is clearly important for its fitness, providing protection against parasites and pathogens. The structure of the immune system is very complex, even in the comparatively simple cases, and the immune defence of an organism is evolving continuously. To fully understand the functions of the immune defence, not only must mechanistic factors in the organism be considered, but also ecological factors that may affect the immune system. The combination of information from both ecological and immunological fields is likely to gain a much better understanding of the complexity. The ecological factors influencing the immune system in insects can be divided into four main categories as described below (Schulenburg et al. 2008).

### Parasite interactions

When it comes to parasites, some interactions are likely to influence host immune systems more than others. One example is the coevolution between a parasite and its host. If a parasite species is closely associated to a specific host species for a long period of time, it might adapt to the defences of the host even if these are changing over time. Even though the host might counter-adapt, the parasite is likely to be more successful in its adaptation due to larger population size and shorter generation time (Hamilton et al. 1990). A host may also be forced to deal with many different parasite species, which requires both diversity and flexibility in the immune system. In addition, parasite manipulation of the defence reaction in the host is also an important factor affecting the immune system.

### Intraspecific constraints

To have an effective immune system is costly for all organisms, which can lead to physiological constraints. Immunity costs result in trade-offs between immunity and other traits that are fitness-related. Probably the most important trade-off is the one between reproductive rate and immunity (Schulenburg et al. 2008). Another constraint originates from Bateman's principle (Clutton-Brock 1988), which claims that females increase their fitness by longevity as their reproductive effort is higher, compared to males who gain fitness by increasing their mating success. Based on this, females should invest more than males in immunity (Rolff 2002).

### Population genetics

In the evolution of host-parasite interactions and thus also the evolution of the immune system of the host, population genetic characteristics are of major importance. These include migration, population size, the frequency of mutations, the structure of metapopulations and the number of genes involved in trait expression (Schulenburg et al. 2008).

### Additional factors

There are several additional factors that might have effect on the insect immune system. These can be both biotic; such as interactions with predators, prey or competitors, and abiotic; for example temperature shift. In a study performed concerning the environmental influence on mosquito immune resistance, it was shown that food availability altered the resistance in the mosquito *Anopheles stephensi* towards the malaria parasite *Plasmodium yoelii yoelii* (Lambrechts et al. 2006). In another study temperature was shown to have a dramatic effect on parasite resistance in the water flea *Daphnia magna* (Mitchell et al. 2005).

### *The immune response in insects*

The immune system in insects consists of several different defence mechanisms, including cellular and humoral defense responses. Cellular defenses are directly mediated by cells called hemocytes (Giglio et al. 2008; Strand 2008). These are found in the hemolymph and can be described as the insect blood cells. Hemocytes have been studied mainly in Diptera and Lepidoptera, where many different classes have been characterized morphologically and functionally. In coleopteran species, however, little is still known in this particular field (Manachini et al. 2010).

Two mechanisms directly mediated by hemocytes are phagocytosis and encapsulation. Phagocytosis is a process where specialized cells ingest and destroy small invaders. If an intruding object is too large to be phagocytosed, a common immune response is instead encapsulation. In this process the hemocytes aggregate to form a capsule around the foreign object, for example a parasitoid egg or a nematode. The encapsulation usually begins 4-6 hours after the parasitism and is completed after approximately 48 hours (Wertheim et al. 2005). The hemocytes activated in the encapsulation process also produce melanin, which leads to a localized blackening of the tissue at the wound site and around the encapsulated object (Pham and Schneider, 2008). When melanin is deposited the capsule also hardens, as a result of several biochemical reactions, leading to the death of the enclosed intruder (Ojala et al. 2005; Mikkola & Rantala 2010). The melanisation process is a conserved immune response in insects, with phenol oxidase (PO) as the key enzyme (Rolff & Siva-Jothy 2004).

Even though encapsulation often is a successful defence against parasitoids, many parasitoid species have developed adaptations to counter this process. Examples of such adaptations are the active destruction of immune cells in the host to disrupt capsule formation, or molecular mimicry of the host. Some species can even survive being encapsulated, by modifying the encapsulation response itself. Many parasitic tachinids (true flies) develop inside the host but penetrate the cuticle or trachea of the host to respire. If encapsulated by host hemocytes or epidermal cells, the parasitoid larva is not killed as long as it can still respire (Strand & Pech 1995).

In addition, parasites and parasitoids are expected to have an evolutionary advantage relative to their hosts, due to short generation times, large populations and high mutation rates, thus overcoming new resistance strategies of the hosts quickly (Gandon 2002). However, there is a great variability in the outcome of empirical studies concerning local adaptation in host-parasite interactions (Cogni & Futuyma 2009). In many cases, the parasites show local adaptation, but in other studies there seem to be no local adaptation or even maladaptation of the parasites (Greischar & Koskella 2007).

#### *The effect of host plant quality on insect immune response*

As mentioned earlier, plant quality is of major importance to herbivorous insects. Host plant quality affects the fecundity of insects as well as their reproductive strategies, such as the

allocation of resources to eggs, size and quality of the eggs, and the choice of sites for oviposition (Awmack & Leather 2002). The quality of the host plant also has effect on insect immune response to parasitoid attacks. If the host plant has high nutritional value, herbivores feeding on it are likely to have resources enough both for egg production and a successful immune response. However, as the defense mechanisms in the plant often are triggered by herbivory, feeding insects might lower the nutritional value and raise the toxicity in the plant. This often leads to an impoverished immune response in the insects with a reduced capacity for egg-encapsulation, as the insects need to allocate more resources for growth or to overcome high toxic levels. In some cases, though, herbivore insects may adapt and instead take advantage of the toxicity of their host plants. The insects can use the defensive metabolites produced by the plants in order to protect themselves from natural enemies (Ojala et al. 2005). This is true for insect herbivores of different orders: Many lepidopterans may sequester iridoid glycosides, aphids may sequester glucosinolates from *Brassica*, and pyrrolizidine alkaloids can be sequestered by chrysomelid beetles (Awmack & Leather 2002).

One example of a herbivore insect adapted to the defense mechanism of the host plant is seen in the European pine sawfly, *Neodiprion sertifer* (Fig. 4), using Scots pine (*Pinus sylvestris*) as its host (Björkman & Larsson 1991). The pine trees contain terpenes, mainly monoterpenes, which in high concentrations can be toxic to insects. The resins in the pine needles function as a defense against herbivores. *Neodiprion sertifer* is affected by these toxic resins to some extent. If the sawfly larvae feed on pine needles with high concentrations of resin acids, the mortality in the early instars is higher and the development slower, compared to larvae feeding on needles with low concentrations of resin acids. However, late instar larvae are not negatively affected by higher concentrations (Larsson et al. 1986). The sawfly larvae are able to cope with the toxins and in turn use them as a deterrent to predators or parasitoids. They can sequester the toxic resins directly when feeding and store it in sacs connected to the mouth. If attacked by a predator or a parasitoid, the larvae immediately flip their heads backwards and start waving them unisonally to scare the enemy away. In addition to this, the larvae can excrete the contents of their sacs (Fig. 5), producing sticky and smelly droplets (Eisner et al. 1974).



**Figure 4.** A parasitic wasp ready to attack a pine sawfly larva.

Photo: Christer Björkman



**Figure 5.** Defense mechanism in the pine sawfly larvae.

Photo: Christer Björkman

## My PhD project

The aim of my research project is to investigate interactions and possible coevolution in tritrophic systems. I will focus on the interactions between the herbal plant purple loosestrife (*Lythrum salicaria*), two beetle species (*Galerucella pusilla* and *Galerucella californiensis*) using purple loosestrife as host plant, and a parasitic wasp (*Asecodes lucens*) attacking both beetle species (Fig. 6). Other species of *Galerucella* with different host plants and other species of *Asecodes* will also be included as comparative studies in parts of the project.

### *The study species*

#### Lythrum salicaria

Purple loosestrife (*Lythrum salicaria*) is a perennial herb, growing in moist or coastal areas. The plant is erect, usually growing to a height of 60-120 cm, with lanceolate leaves and reddish purple or pink flowers. It is common in wetland sites throughout Europe, in North Africa and in western and northern Asia. It has also been introduced in New Zealand and in North America, where it is now by many considered a threat to the native biological diversity (Blossey et al. 2000). In Sweden, purple loosestrife is common throughout the country in the south, whereas in the north it grows almost exclusively in the coastal area. A number of insect species are interacting with purple loosestrife, several of them using the plant both as a food resource and as a host for their offspring (Matos & Obrycki 2007; Hunt-Joshi & Blossey 2005).



**Figure 6.** Some of the study species. Pictures from *UppsalaAnimation*.

### Galerucella

*Galerucella* are leaf-feeding beetles of the *Chrysomelidae* family. There are several different species of *Galerucella* that are using different species of host plants. Some have just one specific host plant species whereas others are more generalistic. Both *Galerucella pusilla* and *Galerucella californiensis* use purple loosestrife as their only host plant, both for feeding and oviposition. The two beetle species are closely related and have similar life cycles. The adult beetles are nearly identical and can be very hard to tell apart in the field, whereas the larvae are quite easily distinguished, especially in the late larval stage. Larvae of *G. californiensis* usually have a bright yellow, almost orange color while *G. pusilla* have a pale white-yellow tone ( Fig. 7). Also the pupae differ in color.



**Figure 7.** Larva of *G. californiensis* (top) and *G. pusilla* (bottom).

Photo: Peter Hambäck

The beetles over-winter as adults and emerge during spring. Mating takes place on purple loosestrife plants. The eggs are deposited directly on the leaves in early summer and hatch after a few weeks. Both larvae and adults feed on the plant, which can sometimes lead to quite severe damage. After 3-4 weeks the larvae pupate in the ground and the new adults emerge from the pupae 2-3 weeks later (Hambäck 2004).



Other species of *Galerucella*, using other host plants than purple loosestrife, will also be included in parts of this project. These are: *G. nymphaeae*, feeding on water lily species like *Nuphar sp.* and *Nymphaeae sp.*, *G. lineola*, using different species of willow (*Salix*) as host plants, *G. tenella*, which primarily use Meadowsweet (*Filipendula ulmaria*) as its host, but also strawberry and other Rosaceae species, and *G. sagittariae*, also using strawberry, as well as other species from the Rosaceae and Primulaceae families as host plants. *G. lineola*, *G. tenella* and *G. sagittariae* are all parasitized by *Asecodes* spp, whereas *G. nymphaeae* is not known to be parasitized at all.

#### Asecodes lucens

*Asecodes lucens* is a small (<1mm) parasitic wasp of the *Hymenoptera* family. It is known to attack *G. pusilla* and *G. californiensis* and can cause a high level of mortality in both species. The parasitoid attacks the beetles in the larval stage, laying one or more eggs inside the larva. When the eggs hatch, the parasitoid larvae start consuming the interior of the host (Fig. 8). Parasitized larvae develop normally until pupation, when they are unable to form normal pupae. Instead the larvae turn into black mummies (Fig. 9) from which the adult parasitoids hatch later (Hambäck 2004; Stenberg & Hambäck 2010).



**Figure 8.** Dissected *G. californiensis*, almost entirely consumed by parasitoid larvae.

Photo: Robert Markus



**Figure 9.** Parasitized, mummified larva of *G. californiensis*, with parasitoid pupae inside.

Photo: Robert Markus

### *Project description*

Previous studies of purple loosestrife in Sweden have found that there is a striking latitudinal variability in the insect community associated to the plant (Hambäck 2004). This project will use a natural gradient from north to south in Sweden, encompassing several localities of purple loosestrife. The aim is to study evolutionary responses in the plant-herbivore-parasitoid interactions and try to detect and disentangle potential differences along this gradient.

One part of the project will focus on the interactions between purple loosestrife and the *Galerucella* beetles and how these interactions might vary between different localities due to local adaptation. Host search in the beetles will be investigated, involving quantifications of secondary chemistry. This will be done by collecting volatiles emitted from the host plant as well as from the beetles themselves, using SPME-technique\* (Solid phase microextraction). Both artificially damaged plants and plants damaged by feeding beetles will be used. The volatile compounds will be isolated and analysed using GCMS\* (Gas chromatography- mass spectrometry). If possible, the results will also be linked to the host search in the parasitoid, as volatiles are known to be used both by *Galerucella* spp and *Asecodes lucens* to locate hosts.

Another part of the project will focus on the immune response in the beetles towards the parasitoid. The aim is to find out more about the structure of the immune system in *Galerucella*, as there is still little information concerning the immunology in coleopteran species. One approach to detect potential differences in the immune response between beetle populations from different areas is to study the hemocyte composition in *Galerucella* larvae and to compare the hemocytes of infested and non-infested larvae. This will be done by performing controlled parasitoid-experiments in the lab, using *Galerucella* from different localities.

\*For information on the techniques see for example *Handbook of Solid Phase microextraction*, ed. Janusz Pawliszyn and *Methods in chemical ecology*, ed. Millar & Haynes.

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