Searching for food in complex environments
Integrating processes at multiple spatial scales

Thomas Alexander Verschut

Academic dissertation for the Degree of Doctor of Philosophy in Plant Ecology at Stockholm University to be publicly defended on Friday 2 June 2017 at 09.30 in Vivi Täckholmsalen (Q-salen), NPQ-huset, Svante Arrhenius väg 20.

Abstract
Resources are often unevenly distributed through the environment, resulting in a challenging task for insects to locate food, mates and oviposition sites. Consequently, there is an ongoing need to unravel how insects rely on behavioural and sensory traits while searching for resources in heterogeneous environments. In the first part of this thesis, I addressed this issue by studying how neighbouring resources can affect the likelihood of insects finding their preferred host resources. These effects of neighbouring resources are commonly referred to as associational effects, and are expected to result from limitations in the sensory physiology of insects. Such limitations constrain the insect’s ability to correctly evaluate resource quality at the different steps involved in insect search behaviour. Furthermore, I determined whether the physiological state of an insect, and sensory experiences made during larval stages, can affect host search behaviour in heterogeneous environments.

By comparing the behaviour of Drosophila melanogaster in environments with single and multiple resources, I found that the presence of neighbouring resources increased the selection rates for attractive resources, while it decreased the selection rates for less attractive resources. These effects are referred to as associational susceptibility and associational resistance respectively. Furthermore, by studying oviposition behaviour, I found that during these small-scale behavioural decisions, associational effects are mainly governed by gustatory mediated selection and less by olfactory mediated selection. The oviposition assay eliminated potential misinterpretations of resource quality along the different steps of search behaviour, hence the results suggested that associational effects rely on distinctive selection behaviour between resource types rather than on sensory constraints.

In the second part of this thesis I determined whether natal experiences can be used by insects as sensory shortcuts to find host resources, and whether this leads to better larval performance on those selected host resources. For this purpose, I studied the interactions between the larval parasitoid Asecodes lucens and the oligophagous leaf beetle Galerucella sagittariae. The results showed that the relationship between oviposition preference and larval performance, of both insect species, depends on an interactive effect between the insects’ natal origin and the quality of the different host resources. Moreover, I found that the natal origin was a better predictor for the adult host preference, rather than for larval performance. This suggests that, aside from the actual quality of the host resources, locating any suitable host might be even more limiting for the female’s fitness.

Keywords: Associational effects, Natal experiences, Olfaction, Oviposition.

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Sammanfattning

En utmaning för insekter i naturliga miljöer är att hitta viktiga resurser, såsom föda eller partner, eftersom dessa resurser ofta är ojämnt fördelade i landskapet. För att förstå de mekanismer som möjliggör för insekter att möta denna utmaning behöver vi förstå de beteenden och sinnesintryck som används för att hitta resurser i komplexa miljöer. I första delen av min avhandling har jag studerat hur komplexitet i form av alternativa resurser påverkar sannolikheten att en insekt finner sin prefererade resurs. Effekter av alternativa resurser i grannskapet benämnas ofta associativa effekter, och beror på begränsningar i insekters sinnesfysiologi. Dylika begränsningar påverkar insekters förmåga att utvärdera kvaliteten på resurserna i ett område. Eftersom den förmågan är en fundamental aspekt av en insekts sökprocess så beslutade jag mig för att studera hur en individs fysiologiska tillstånd och tidigare erfarenheter under larvstadiet påverkar sökbeteendet i heterogena miljöer.


List of Publications

This thesis is based on the following papers, which are referred to by their roman numerals:


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Introduction

One of the main goals of community ecology is to examine processes underlying species distribution patterns [1-3]. While processes at local and regional scales were traditionally assumed to affect the distribution of species independently [4-6], a hand-full of trait-based theories have popularized the idea that these patterns are simultaneously influenced by processes occurring at different spatial scales [7-10]. While these approaches have helped silencing the much criticized lack of general principles in community ecology [11, 12], there is still a need for a mechanistic understanding of how behavioural traits can generate responses to environmental heterogeneity. During the last decades, the remarkable diversity of morphological and physiological traits found among insect taxa has offered ecologists an intriguing system to study the mechanisms underlying community composition [13, 14]. However, the immediate drawback of this high diversity is that it has been difficult to connect the observed species distribution patterns to general principles among taxa. A way to unravel these patterns, would be by studying how specific traits are involved in the responses of insects to environmental heterogeneity [13, 15]. Although several studies have been able to link dispersal capacities [15, 16], host specialization [17, 18], and sensory modalities [19, 20], to species distribution patterns, these studies have concurrently shown that we are still far from a holistic understanding of how insects respond to environmental heterogeneity.

It is well recognized that environmental heterogeneity is a major constraint on the ability of many insects species to find food, mates and oviposition sites [21, 22]. To find suitable resources, insects usually follow a multi-step process, in which they have to locate suitable habitat patches, then needs to distinguish hosts from non-hosts within the patch, and finally needs to select the most suitable resource among the potential hosts [23-25]. The sensory information used to locate a suitable habitat patch is often not of high enough resolution to distinguish between the different resources within the habitat patch. It is expected that insects solve this problem by integrating multiple sources of sensory information while evaluating resource quality at each step of the search behaviour process. The first steps of search behaviour are often referred to as pre-alighting behaviour, during which insects mainly use long-range sensory information, such as olfactory and visual cues. The final steps of insect search behaviour are referred to as post-alighting selection behaviour, during which the insects use additional short-range sensory information, like mechanosensory or gustatory cues, to evaluate the suitability of the resource [19, 26].
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By comparing the behaviour of *Drosophila melanogaster* in environments with single and multiple resources, I found that the presence of neighbouring resources increased the selection rates for attractive resources, while it decreased the selection rates for less attractive resources. These effects are referred to as associational susceptibility and associational resistance respectively. Furthermore, by studying oviposition behaviour, I found that during these small-scale behavioural decisions, associational effects are mainly governed by gustatory mediated selection and less by olfactory mediated selection. The oviposition assay eliminated potential misinterpretations of resource quality along the different steps of search behaviour, hence the results suggested that associational effects rely on distinctive selection behaviour between resource types rather than on sensory constraints.

In the second part of this thesis I determined whether natal experiences can be used by insects as sensory shortcuts to find host resources, and whether this leads to better larval performance on those selected host resources. For this purpose, I studied the interactions between the larval parasitoid *Asecodes lucens* and the oligophagous leaf beetle *Galerucella sagittariae*. The results showed that the relationship between oviposition preference and larval performance, of both insect species, depends on an interactive effect between the insects’ natal origin and the quality of the different host resources. Moreover, I found that the natal origin was a better predictor for the adult host preference, rather than for larval performance. This suggests that, aside from the actual quality of the host resources, locating any suitable host might be even more limiting for the female’s fitness.

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The most commonly used sources of pre-alighting sensory information are visual and olfactory cues. The distribution of different light receptors throughout the compound eyes allows insects to use structures in the environment for long distance orientation [27], while also maintaining the ability to observe relatively detailed structures at shorter distances [28-30]. Visual cues often provide a more stable source of information than olfactory cues, which instead consist of small volatile molecules that are easily affected by the conditions of the surrounding environment [31-33]. Although most insect species have relatively sensitive sensory receptors [14, 27], locating suitable host resources can still remain a challenging task. The complexity of insect search behaviour is probably best illustrated by a description of how insects use odorants to locate potential resources. Odour plumes usually travel downwind in filamentous plumes where biologically meaningful volatiles are intertwined with puffs of air containing no odours [34]. The probability that an insect can locate the odour source emitting the volatiles is often affected by the meandering plume pattern, caused by obstructions in the habitat and the interference of the wind direction [34, 35]. The zigzagging upwind flight pattern found in moths, is example of how insect have adopted strategic upwind flight patterns to enlarge the probability of keeping track of the odour plume [32-34].

After successfully following the odour plume to the odour source, the insect will evaluate the structure of the resource [36, 37], and the presence of specific olfactory and gustatory cues before actually selecting it [38-41]. This process illustrates the importance of insects being able to separate host from non-host related sensory information while searching for resources. However, limitations of the sensory physiology can cause insects to misinterpret the resource quality at the different search behaviour steps. Through these misinterpretations, neighbouring resources might affect the probability that the insect selects the most suitable host resource within a patch [42-45]. This interference of neighbouring resources is commonly referred to as associational effects. Unfortunately, the intrinsic limitations of testing sensory misinterpretations of resource quality, at the different steps of search behaviour, has made it difficult for ecologists to empirically test the actual mechanisms underlying associational effects [46, 47]. However, a mechanism that can potentially help insects to deal with environmental heterogeneity, are experiences made in the natal habitat. These experiences can serve as sensory shortcuts to locate suitable resources based upon sensory experiences made during the larval stage [48, 49]. Ultimately the oviposition site picked by a female insect can provide the larval stage with information that primes host choice during their adult life stages [48, 49]. In the papers supporting this thesis, we firstly studied how associational effects between alternative resources can affect insect search behaviour, and secondly, how insects can use physiological and sensory adaptations to deal with the multitude of sensory information experienced while searching for resources.
Aim of the thesis

The main purpose of this thesis was to unravel how behavioural and sensory traits underlie insect search behaviour in heterogeneous environments, and how insects use physiological and sensory adaptations to deal with the environmental heterogeneity experienced during search behaviour. In the first part of this thesis, we used the common fruit fly Drosophila melanogaster Meigen (Diptera: Drosophilidae) to study how interactions between sensory traits and environmental heterogeneity can generate associational effect between resources. More specifically, we firstly addressed how resource density and resource frequency, which are two major components of environmental heterogeneity, can result in predictable patterns of associational effects (Paper I). Afterwards, we studied whether mating induced behavioural changes can lead to differences in resource selection rates, and thereby modulate the strength of associational effects (Paper II). Finally, we used fruit flies with sensory deficiencies to investigate the importance of specific sensory modalities in generating associational effects during oviposition site selection (Paper III).

In the second part of this thesis we aimed to determine whether sensory experiences made during larval stages can serve as sensory shortcuts to locate host resources during the adult stages. We used a naturally occurring species interaction, involving the larval parasitoid Asecodes lucens Nees (Hymenoptera: Eulophidae), attacking the leaf beetle Galerucella sagittariae Gyllenhaal (Coleoptera: Chrysomelidae) to determine the consequences of natal origin on resource selection behaviour in heterogeneous environments. Although G. sagittariae lays eggs on several distantly related wetland plant species, the larvae suffer comparable parasitism rates by A. lucens on all host plant species. As unrelated plants are often characterized by distinct chemical profiles, we tried to unravel how the beetle and the parasitoid integrate sensory information to select between the different host resources. Therefore, we collected insects originating from two distinct natal hosts, and determined if natal experiences can serve as sensory shortcuts to select resources from their natal habitat. Subsequently, we determined whether this resource preference leads to better larval performance on the selected host resources (Paper IV).
Associational effects

A potential mechanism that can influence species distribution patterns in heterogeneous environments are associational effects. These effects occur when a neighbouring resource decreases or increases the probability that an insect will select a particular resource within a patch [42, 47]. Associational effects are best illustrated by imagining a patch consisting of host plant species, growing alongside other non-host plant species. When, for example, the odours emitted by neighbouring non-host plants reduce the attraction rate of the host plants, this may lead to an underestimation of host plant density by the insect, causing the non-host plants to provide associational resistance to the host plant. On the other hand, highly attractive odours of non-host plants might lead to an overestimation of host plant density, by which a larger number of insects will distribute themselves along the available host plants, leading to associational susceptibility [43, 50, 51]. A variety of behavioural mechanisms have been hypothesized to generate different patterns of associational effects between resources [52-56]. However, the intrinsic limitations of testing these different hypotheses under natural conditions, has made it difficult for ecologists to reach a consensus on when to expect associational resistance or associational susceptibility [46, 47].

One of the main hypothesis for associational effects proposes that these effects are a consequence of limitations in the sensory physiology of insects, which constrain the insects’ ability to evaluate resource quality at different levels of host search behaviour [52-54]. As explained earlier, search behaviour can be divided into several behavioural steps occurring along different spatial scales, making it likely that the resolution of sensory information used to locate a resource patch might not be sufficient to distinguish between the quality of the individual resources within the patch [47, 53]. This illustrates the importance of understanding how an insect uses its sensory system to interpret the available information along each step of the search behaviour process [42-45]. During the last decade, several studies have helped to popularize the idea that we can use resource perception, or resource attraction rates, to determine when to expect associational resistance or associational susceptibility [46, 47, 57].

The work presented in the first part of this thesis aimed to further develop the idea that we can use resource perception to predict associational effects, using the common fruit fly Drosophila melanogaster Meigen (Diptera: Drosophilidae) as a model organism. One of the benefits of using fruit flies as a model organism is the detailed understanding of the organization of their sensory system [58, 59], which has made it possible to identify which sensory
information is involved in behavioural responses [60-63]. For example, various studies have demonstrated that mating affects the sensitivity of female fruit flies towards specific olfactory cues associated with microorganisms indicating nutrient rich oviposition sites [64-66]. Therefore, the detailed understanding of the sensory biology of fruit flies makes them a particularly useful model organism to study how sensory mechanisms can be responsible for generating associational effects. More specifically, the aims for this part of the thesis were to determine how resource density and resource frequency within a patch can generate associational effects (Paper I), how mating induced changes in resource selection behaviour can modulate associational effects (Paper II), and how specific sensory modalities generate associational effects during oviposition (Paper III).

**Resource density and resource frequency**

The experiments presented in Paper I were designed to determine how resource density and resource frequency, as components of environmental heterogeneity, can generate associational effects between two resources. We built a wind tunnel assay in which we firstly determined the ability of fruit flies to discriminate between odour sources emitting different vinegar concentrations. Subsequently, we determined the effects of resource density and frequency on search behaviour in complex environments by combining two odour sources into various patch arrangements. Finally, we interpreted the search behaviour patterns found in our experiments to determine the role of resource density and frequency in generating associational effects. The starting point for this study was the hypothesis that qualitative differences in long-range resource perception could lead to associational susceptibility for one resource type and associational resistance for the other resource type in heterogeneous patches [46]. To validate this hypothesis, we exposed individual fruit flies in a wind tunnel to four concentrations of balsamic vinegar, and found the strongest qualitative differences in attraction rates between 1% vinegar (high attraction rate) and 0.1% vinegar (low attraction rate).

Afterwards we combine these two vinegar concentrations in patches consisting of four odour point sources releasing either of the two vinegar concentrations. We tested two homogeneous resource patches which only contained odour sources of 0.1% or 1% vinegar, and three heterogeneous resource patches consisting of different frequencies of the two vinegar concentrations. Moreover, we used the cumulative concentration of the four vinegar odour sources within the patch as a substitute for resource density. We exposed the fruit flies in a wind tunnel to odour plumes of these different patch arrangements and they would first have to navigate upwind through the mixed odour plume, before they would be able to select a specific odour source in closer
proximity of the release points. By using the cumulative concentration of all vinegar odour sources within the patch as a substitute for resource density, we found that the attraction to the patches was constrained by an optimum vinegar concentration after which the attraction rate decreased again (Figure 1). Generally, it is expected that higher densities of odour sources cause higher attraction rates, as the strength of an odour plume is intrinsically linked to the number of odour sources within a patch [67]. However, acetic acid, which is one of the key odour compounds in vinegar, has been shown to be of importance in long-range attraction, but has also been found to be involved in short-range aversion [60, 63]. This makes it likely that the plateau observed in the attraction rates to resource density was caused by an ambivalent response to acetic acid.

When we analysed the relative selection rates per individual odour release points we translated the selection rates for the odour sources into associational effects. This analysis showed that the attraction rate of 0.1% vinegar decreased with the decreasing frequency of 0.1% vinegar in the patch (Figure 2). This result represents associational resistance for 0.1% vinegar, as the attraction rate of 0.1% vinegar release points was lowered when both vinegar concentrations were mixed in heterogeneous patches. On the other hand, the attraction rate of 1% vinegar increased with the decreasing frequency of 1% vinegar in the patch. This represents associational susceptibility as the attraction rate of 1% vinegar is higher in the heterogeneous patches. In other words, our results illustrate that 1% vinegar became more susceptible, and 0.1% less

**Figure 1.** The landing rates of *Drosophila melanogaster* on the patches with the different vinegar concentrations ± 95% confidence intervals. The vinegar concentrations are given on a log scale and represent total resource density in the patch. Figure taken from [68].
susceptible, to be selected by the fruit flies in mixed patches than in patches releasing only one of the vinegar concentrations. The outcome of this study suggests that the combination of associational susceptibility and associational resistance actually occurred because the fruit flies used the more attractive vinegar concentration during long-range attraction towards the patch, and for short-range resource selection within the patch. Consequently, when an insect maintains a preference for the more attractive resource within the patch this can lead to a predictable outcome in which the more attractive resource receives a higher number of insects, and experiences associational susceptibility, and the less attractive resource thereby experiences associational resistance.

![Figure 2](image)

**Figure 2.** The attraction rates of 0.1% vinegar (dashed line - light brown points) and 1% vinegar (solid line - dark brown points). On the x-axis, the frequency of 1% vinegar odour sources in the patch is given. The regression lines are given with their 95% confidence interval and the values of selecting (1.00) or not selecting (0.00) an odour source are jittered vertically and horizontally to visualize the binomial data. Figure replotted in inverted direction opposed to the original figure in [68].

**Mating induced behavioural changes**

It is well established that mating affects the sensitivity of female fruit flies towards specific sensory cues associated with oviposition sites [64-66]. Therefore, in paper II we aimed to determine whether the behavioural patterns found in the previous study could be modulated by the physiological state of the insect. In the following experiments we exposed unmated and mated female fruit flies to apple and banana odour sources in the same wind tunnel assay as
described in the previous study. We used fruit odours, rather than the vinegar concentrations used in the previous study, to provide the fruit flies with odours that could derive from actual oviposition sites. As in the previous study, we first tested the qualitative differences of apple and banana in single odour source experiments, and found that banana is more attractive than apple to both unmated and mated fruit flies.

However, when we exposed the fruit flies to apple and banana odours in mixed resource patches, we found strong differences in the behaviour of unmated versus mated fruit flies. More specifically, the unmated fruit flies maintained a preference for banana, which resulted in higher attraction rates for banana with the decreasing frequency of banana in the patch. On the other hand, the attraction rate of apple odour sources decreased when both resources were offered at the same time. These results represent associational susceptibility for banana and associational resistance for apple respectively. In contrast to the behaviour of the unmated fruit flies, we found that the mated fruit flies did not maintain a preference for either resource. Consequently, the resource selection rates were not affected by either the identity of the odour source or by the frequency of the two odour sources in the patch. However, as the mated fruit flies did show long-range attraction to the different patches, we believe that the contrast between these two resources might not have been apparent for the mated fruit flies during short-range resource selection. Moreover, the lack of resource preference by the mated fruit flies actually eliminated the interactions between the two resources and caused no associational effects between apple and banana.

Figure 3. The attraction rates of (a) unmated and (b) mated female fruit flies to apple odour sources (solid lines - red points) and banana odour sources (dashed lines - yellow points). The x-axis represents the frequency of apple and banana odour sources in the patches. The predicted linear regression lines are given with their 95% confidence interval. The values of selecting (1.00) or not selecting (0.00) and odour source are jittered vertically and horizontally for visualization. Figure adapted from [69].
As mentioned before, the starting point of our experiments was the hypothesis that qualitative differences in long-range resource perception could lead to associational susceptibility for one resource type and associational resistance for the other resource type in heterogeneous patches [46]. By working with the unmated and mated fruit flies we had the opportunity to compare how associational effects are generated based on the degree to which the insects distinguish between resources. While it is likely that the mated females used the fruit odours as long-range attractants, they did not detect the necessary short-range differences between apple and banana to select for either of the resources, which resulted in a loss of associational effects. Consequently, both paper I and paper II exemplified the importance of insects maintaining a resource preference, during both long-range and short-range resource selection, for the occurrence of associational effects.

Sensory modalities used during oviposition

As our previous study suggested that losing the ability to detect short-range differences between apple and banana eliminated the occurrence of associational effects, we wanted to determine which other sources of short-range sensory information could instead generate associational effects. Therefore, in paper III we developed an oviposition assay, representing the same environmental heterogeneity as tested in the wind tunnel assays, and compared the oviposition behaviour of wild type flies, with that of fruit flies with olfactory and gustatory deficiencies. We specifically selected olfactory and gustatory deficiencies as these sensory modalities have previously been shown to be of importance for fruit flies during post-alighting resource selection [38-41].

Insect olfactory receptors (ORs) function as a heteromeric complex formed by ligand-binding odorant receptors and a chaperon co-receptor (Orco) that completes the signal transduction cascade [70, 71]. Moreover, the olfactory receptors are complemented by a second family of odorant receptors, the ionotropic glutamate-like receptors (IRs), of which several are dependent on either of the two broadly expressed co-receptors IR8a and IR25a [72, 73]. In our experiments, we included Orco2, IR8a1 and IR25a2 mutant strains to determine the importance of olfaction in generating associational effects during post-alighting resource selection. Due to the ubiquitous expression of Orco in all sensory neurons housing olfactory receptors [71], Orco2 mutants can be assumed to have stronger sensory deficiencies than the IR8a1 and IR25a2 mutant flies, which respectively only lack dedicated ionotropic co-receptors for the detection of certain carboxylic acids and amines respectively [72, 73].
Most of the insect gustatory receptors are housed in sensilla on the labellum, anterior wing margins, leg tarsi and the ovipositor of the female [74, 75]. To determine the degree to which gustatory cues are involved in generating associational effects we studied the behaviour of four different Pox-neuro (Poxn) mutant strains, differing in the functional reduction of their sensory capacities. The Poxn mutation turns all poly-innervated gustatory bristles into mono-innervated mechanosensory bristles, eliminating the function of all direct-contact gustatory sensilla in these mutant strains [76-79].

![Image](image.png)

**Figure 4.** The top of the figure represents the oviposition assay used to compare the oviposition behaviour of wild type flies (w1118) with that of fruit flies with olfactory deficiencies (Orco) and gustatory deficiencies (Poxn). The illustration gives an example of a patch consisting of two apple (red) and two banana (yellow) oviposition discs. These graphs show the eggs distribution and the linear regression lines with their 95% confidence intervals for apple and banana oviposition substrates. The points representing egg distribution are jittered vertically and horizontally for visualization purposes.

In our oviposition experiment we found that wild type fruit flies strongly prefer to oviposit on apple rather than on banana substrates, and that the overall oviposition rate is positively affected by the increasing frequency of banana in the patch (Figure 4). When translating these results into associational effects we can say that the increasing oviposition rate on apple with the decreasing frequency of this resource in the patch signifies associational susceptibility. On the other hand, the decreasing oviposition rate on banana with the decreasing frequency of banana in the patch signifies associational resistance. Out of the three olfactory mutants, we only found that the oviposition behaviour of the Orco flies differed from the wild type flies. This difference mainly occurred because the oviposition rate of the Orco flies on apple increased less with the decreasing frequency of this resource than the oviposition rate on banana, suggesting that the strength of associational effects between apple and
banana was weaker in the experiments with Orco² flies compared to the wild type flies. We found the strongest change in associational effects in the experiments with the Poxn mutants, which lack the ability to interpret gustatory cues by direct contact with the substrate. The oviposition behaviour of these flies only showed small differences between the apple and banana substrates and only a weak effect of resource frequency (Figure 4). Therefore, these results suggest that neither of the resources experienced any associational effects in the experiments with the Poxn mutant flies. In conclusion, these results show that the occurrence of associational effects mainly relied on the use of non-volatile chemical cues, and less on the use of volatile cues, during oviposition resource selection.

Conclusion

One of the main hypothesis on the occurrence of associational effects is that these effects typically occur due to limitations in the sensory physiology of insects, which constrain their ability to evaluate resource quality at different levels of host search behaviour [52-54]. As the fruit flies gave us the possibility to observe these hierarchical decisions within relatively small experimental setups, they served as a perfect model organism to test how resource heterogeneity leads to associational effects. Our experiments presented, to our knowledge, some of the first evidence showing that sensory constraints in evaluating resource quality is not necessarily a mechanism generating associational effects. Instead, our results show that these effects can also be generated through preferences for specific resources. The work presented in the first two studies suggests that similar effects may arise whenever the insect distinctively selects between different resources during long-range and short-range pre-alighting search behaviour. This effect is perhaps best illustrated by the results we found for mated fruit flies, which used the fruit odours for long-range attraction, but did not discriminate between the two odour sources during short-range resource selection. As a result, these fruit flies did not generate any associational effects between the two odour sources. Furthermore, during post-alighting resource selection, which we tested in the oviposition experiments, we found that sensory deficiencies result in different behavioural patterns compared to those found for the wild type fruit flies. The sensory deficiencies either modulated the strength of associational effects between the two resources, or when the insect could not distinguish between the two resources, entirely eliminated the occurrence of associational effects.
Host preference and larval performance

The oviposition preference and larval performance relationship is an evolutionary mechanism that has been suggested to restrict the host use of generalist insects. The hypothesis for this relationship states that females maximize their fitness by preferably selecting host species with the highest quality for progeny development [80-82]. Although numerous empirical studies have supported this relationship [83-85], a comparable number of studies have failed to provide compelling evidence [85-87]. Consequently, the extent to which host use is constrained by this relationship remains open for debate. Recent studies have led to the idea that the lack of such a relationship cannot be explained by absence of an interaction between female preference and host plant suitability [85], but is most likely the result of additional physiological and environmental factors [88-90]. However, one of the physiological factors that could possibly strengthen the interaction between host preference and host suitability are natal experiences, through which sensory experiences made during the larval stage serve as sensory shortcuts to locate suitable resources during adult stages [48]. Ultimately this effect means that the oviposition site picked by an insect will provide the larval stage with sensory experiences that prime the host plant choice during their adult life stages [91, 92].

On the other hand, a factor that could simultaneously weaken the oviposition preference and larval performance relationship is the pressure of natural enemies. For example, it is likely that enhanced larval development also increases the likelihood that natural enemies, like larval parasitoids, will attack these high-quality prey larvae [93, 94]. Larval parasitoids are a diverse group of natural enemies of which the progeny develops within larvae of other arthropod species and unconditionally causes the death of the host [95, 96]. Considering that larval parasitoids are obligated to develop within the body of a single host, they strongly benefit from large and fast growing hosts [96-98]. A consequence of this close interaction is that there is often a correlation between the sensory information used by both species during host search behaviour [99, 100]. Therefore, the transfer of sensory information along the different trophic levels might serve as an additional factor influencing the relationship between oviposition preference and larval performance [24]. The experiments presented in this part of the thesis were designed to determine whether sensory experiences made by the herbivore and larval parasitoid, during the larval stages, can affect host search behaviour during the adult stages.
We used a tritrophic system consisting of the larval parasitoid *Asecodes lucens* Nees (Hymenoptera: Eulophidae), attacking the leaf beetle *Galerucella sagittatae* Gyllenhaal (Coleoptera: Chrysomelidae), which is commonly found on two unrelated host plants; *Potentilla palustris* (L.) Scop. (Rosaceae) and *Lysimachia thyrsiflora* (L.) (Primulaceae). It has been observed that the beetle larvae suffer comparable parasitism rates on both host plant species. As unrelated plants often have quite distinct chemical profiles, we tried to unravel how the beetle and the parasitoid integrate sensory information to select between the different host resources. Both of the host plant species are commonly found along marshy riversides and in wetlands in central Sweden, and the adult beetles overwinter in the soil litter layer of these habitats. Around mid-May the host plant growing season starts, and the overwintering adults will lay egg batches on the underside of the leaves until the beginning of July. The larvae hatch approximately after two weeks and feed upon the leaves until they pupate about three weeks later. In this period both larvae and adults can be found to feed upon the same host plants, but by the end of July all adults are of the new generation [101, 102]. During the early developmental stages, the larvae run the risk of being attacked by the parasitoid, which lays several eggs in the body of the larvae, and later transforms the infected larvae in hardened black mummies. The parasitoids normally overwinter as pupae within the mummified host larvae [103, 104], but for unknown reasons a considerable number of adult parasitoids already hatch at the end of the season in which the brood was laid [101, 105].

**Figure 5.** Conceptual diagram illustrating the tritrophic system. We collected *Galerucella sagittatae* and *Asecodes lucens* from *Potentilla palustris* and *Lysimachia thyrsiflora* dominated locations. We used these insects to study different behavioural components, like the olfactory preferences and antennal responses of the beetle to odours of both host plants (grey odour plumes). Afterwards, we studied the oviposition preference for either of the host plants by the beetles, and measured the performance of the larvae on both host plants. Finally, we studied the olfactory preferences of the parasitoids and measured the performance of the parasitoid larvae on host larvae from both host plants. Figure adapted from [101].
Natal origin in tritrophic interactions

We first collected both insect species at natural populations dominated by either *Lysimachia* or *Potentilla* host plants, and determined whether sensory experiences made during the larval stages affected their preference for a specific host species. In these experiments we released adult insects individually into small olfactometers where they could exclusively select their preferred host based upon the odours directly deriving from the host plants, or from beetle larvae on the host plants respectively. In the next experiment, we aimed to explain the observed behavioural responses by identifying the volatile organic compounds emitted by the host plants, and performed chromatographic-electroantennographic detection to identify which of the released volatiles caused antennal responses in the beetles. Finally, we determined whether the natal origin of both insect species affected the performance of their larvae on either of the two alternative hosts. To answer this question, we performed cage experiments in which the beetles could oviposit on their preferred host plant species. Once the oviposition cycle was finished, we collected egg batches and monitored the development of the larvae. For the parasitoids we collected late instar beetle larvae from various field locations and maintained them until pupation. We used the parasitoids that emerged from the mummified larvae to quantify larval performance by determining the sex ratios and by measuring the length of the hind tibia of each individual.

Our experiments showed that the relationship between oviposition preference and larval performance, of both insect species, is influenced by an interaction between natal origin and the quality of the different host resources. However, the proposed interactions resulted in differences among the natal origins and insect species. Only the natal *Lysimachia* beetles maintained an olfactory and oviposition preference for their natal host plant, while also having higher larval performance on the natal host plant. The natal *Potentilla* females, on the other hand, showed no preference for their natal host plant and also had lowered larval performance on the natal host plant (Figure 6). However, while the larval growth rates of both natal *Lysimachia* and natal *Potentilla* beetles were higher on their natal host plant, the final pupal weights were always higher on *L. thyrsiflora* than on *P. palustris*, indicating a developmental advantage for larvae on this host plant (Figure 7).
For the parasitoids, we found strong effects of natal origin on the preference towards odours deriving from their natal host larvae (Figure 6). Despite the fact that we did not find any evidence that the length of the mummified larvae, or the brood size of the parasitoids, differed between both natal origins, we did find a slight fitness advantage for those parasitoids hatching from natal *Lysimachia* larvae. Firstly, the sex ratio from broods originating from *Lysimachia* larvae was strongly biased towards females compared to broods originating from *Potentilla* larvae. Secondly, the hind tibia length of the parasitoids originating from *Lysimachia* larvae were longer, and indicated larger body sizes of the adult parasitoids (Figure 7). Various studies have shown that parasitoids often allocate more female than male offspring to larger hosts as female offspring is more valuable in terms of reproductive output [106, 107]. Moreover, as larger body size often correlates with the survival for larval parasitoids [96-98], the results of two reproductive fitness measures suggest an advantage for female parasitoids to lay their eggs in natal *Lysimachia* larvae rather than in natal *Potentilla* larvae.

Figure 6. Behavioural responses by (a) natal *Potentilla* and natal *Lysimachia* beetles and by (b) natal *Potentilla* and natal *Lysimachia* parasitoids to odour profiles deriving from the two alternative hosts. Figure adapted from [101].

Figure 7. Larval performance illustrated by (a) the pupal weight of natal *Potentilla* beetles and natal *Lysimachia* beetles on both host plants and (b) hind tibia length for female and male *Asecodes lucens* hatching from *Galerucella* larvae from *Potentilla palustris* and *Lysimachia thyrsiflora*. Figure adapted from [101].
Conclusion

We only found a positive relationship between oviposition preference and larval performance for natal *Lysimachia* beetles and not for the natal *Potentilla* beetles or for the parasitoids. Moreover, the natal origin was often a better predictor for adult host preference than for larval performance, which suggests that locating any host might be more limiting for the females than the actual quality of the selected host resource. The outcome of our experiments exemplified the complexity of these relationships in natural systems, and suggests the importance of additional factors influencing the interaction between resource quality and larval performance. This is especially shown by the parasitoids for which the natal origin strongly affected their observed behavioural responses, but did not strengthen the relationship with larval performance.

Our results can be placed in a broader evolutionary perspective by comparing them with other tritrophic systems. As an example, various leaf beetle species have been found to use host plants with high levels of defence chemicals, when these plants also provide lower parasitism pressure [108-110]. Although our study was not specifically designed to test this possibility, our results give us the opportunity to contemplate about the effects of natal origin on host search behaviour of our species in heterogeneous environments. For example, the strongly biased host selection behaviour of the parasitoids for host larvae of their natal origin would make it beneficial for the beetles to actually use multiple host plant species. If we assume that natal *Lysimachia* parasitoids maintained a strongly biased host selection over multiple generations, this high parasitism pressure may have caused the beetles to start using lower quality alternatives instead [108-111]. This hypothesis could explain why the difference in larval performance was not strong enough to prevent natal *Potentilla* beetles from using lower quality host plants, and why they also responded to odours from *L. thyrsiflora*.

Overall, our results show that by incorporating information on host preference and larval performance while studying tritrophic systems, we can improve our understanding of the observed host search behaviour. Moreover, the results show that interactions between the different trophic levels can serve as possible explanations why insects adapt to lower quality resources. Studying such interactions can improve our understanding of the evolutionary consequences of species interactions [81], and help understand potential host race formation or sympatric speciation [112-114].
Concluding remarks

The work presented in this thesis aimed to unravel the importance of behavioural and sensory traits in insect search behaviour. Before concluding this thesis, I would like to thank Peter Hambäck and Peter Anderson for their guidance and supervision in developing the scientific backbone of this thesis. I believe that we have managed to incorporate ideas originating from different scientific areas into appealing experimental studies. I would also like to thank everybody who has contributed as co-author, or with ideas and support in developing this thesis.

The experiments presented in this thesis relied on the groundwork laid by chemical and molecular research to identify how insects integrate complex sensory information while searching for resources [115, 116]. Our studies showed that resource preference can lead to predictable patterns of associational effects between resources. Consequently, resource preference can have strong effects on the distribution of insects among different resources in heterogeneous environments. Moreover, our results also showed that the relationship between oviposition preference and larval performance depends on interactions between sensory experiences and the quality of potential host resources. Overall, the work presented in this thesis can be seen as an advocacy for future studies to recognize the importance of behavioural and sensory traits in the responses of insects to environmental heterogeneity.

Recent developments in trait-based ecology have shown how plant traits can be used as predictors of community assembly [117, 118], and have increased the interest of other research areas in using trait-based approaches [119, 120]. With the advances made in chemical and molecular research, behavioural ecologists now have the opportunity to identify basic behavioural and sensory traits, and should use this opportunity to reach a consensus on how these traits may underlie community assembly processes. For example, if we can determine whether associational effects result from an interaction between the consumer organisms and their focal resources, or from multiple interactions with different resources within a patch, we can fully disentangle the mechanisms underlying these effects. This exemplifies the need of combining behavioural experiments, with chemical analysis to determine which sensory information, or which changes in the use of sensory information, can serve as predictable signals of resource use in heterogeneous environments. Eventually, such knowledge can be used to achieve a better understanding of the evolutionary ecology of insect-host interactions, and help the development of biological control strategies exploiting insect host search behaviour.
References


